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Lucía Prieto Torres

Linfomas cutáneos con expresión de CD30. Clasificación, pronóstico y terapias dirigidas

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LINFOMAS CUTÁNEOS CON EXPRESIÓN DE CD30. CLASIFICACIÓN, PRONÓSTICO Y TERAPIAS DIRIGIDAS.

CUTANEOUS LYMPHOMAS WITH CD3O EXPRESSION. CLASSIFICATION, PROGNOSIS AND TARGETED THERAPIES.

LÍNEA DE INVESTIGACIÓN: CÁNCER. NEOPLASIAS HEMATOLÓGICAS Y CUTÁNEAS

Autor: Lucía Prieto Torres

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Memoria para optar al grado de Doctor en Medicina por compendio de Publicaciones

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Septiembre de 2019

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CERTIFICA:

Que el trabajo titulado “LINFOMAS CUTÁNEOS CON EXPRESIÓN DE CD30. CLASIFICACIÓN, PRONÓSTICO Y TERAPIAS DIRIGIDAS”, que presenta Dña Lucía Prieto Torres ha sido realizado bajo mi dirección y reúne, a mi juicio, originalidad y contenidos suficientes para que sea presentado ante el Tribunal correspondiente y optar al grado de Doctor por la Universidad de Zaragoza.

Y para que así conste y a los efectos oportunos, expedimos el presente certificado en Zaragoza a de de 2019.

Fdo. Dr. Mariano Ara Martín

Dña. Socorro María Rodríguez Pinilla, FEA del Servicio de Anatomía Patológica de la Fundación Jiménez Díaz y Profesora titular del Departamento de Anatomía Patológica de la Universidad Autónoma de Madrid,

CERTIFICA:

Que el trabajo titulado "LINFOMAS CUTÁNEOS CON EXPRESIÓN DE CD30. CLASIFICACIÓN, PRONÓSTICO Y TERAPIAS DIRIGIDAS", que presenta Dña Lucía Prieto Torres ha sido realizado bajo mi dirección y reúne, a mi juicio, originalidad y contenidos suficientes para que sea presentado ante el Tribunal correspondiente y optar al grado de Doctor por la Universidad de Zaragoza.

Y para que así conste y a los efectos oportunos, expedimos el presente certificado en Zaragoza a de de 2019.

Fdo. Dra Socorro María Rodríguez Pinilla

“No siempre podemos hacer grandes cosas, pero si que podemos hacer cosas pequeñas con gran amor”

S. Teresa de C.

A mi familia.

Lucía Prieto Torres

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RESUMEN

La expresión de CD30 en linfocitos tumorales ha cobrado importancia desde la aparición de Brentuximab Vedotin (BV), un fármaco que utiliza este receptor como diana terapéutica y ha demostrado resultados prometedores en muchos pacientes. La expresión de este marcador no es infrecuente en los linfomas cutáneos tanto B como T, y no ha sido por el momento estudiada en profundidad en muchos de ellos.

Esta tesis tiene como objetivo revisar aspectos de la clasificación de los linfomas cutáneos, tanto clínica como fenotípica y molecular, centrándonos en algunos cuya expresión de CD30 resulta significativa y poco estudiada hasta la fecha y en los que estas nuevas características fenotípicas y moleculares pueden ser relevantes para una mejor clasificación pronóstica y terapéutica de los pacientes.

En el primer trabajo realizamos una revisión general de las alteraciones géneticas, epigenéticas y moleculares más relevantes descritas en procesos linfoproliferativos T CD30 positivos primarios cutáneos hasta la fecha y su posible potencial como dianas terapéuticas.

El segundo trabajo identifica características morfológicas e inmunofenotípicas que ayudan a reconocer linfomas anaplásicos con reordenamientos de DUSP-22, identificando como características comunes en éstos la presencia de marcadores T y la ausencia de marcadores citotóxicos y de la vía de JAK/STAT.

En el tercer trabajo describimos una serie de 9 pacientes con lesiones linfoproliferativas CD30+ y EBV+ que amplían el espectro clínico-patológico de la úlcera mucocutánea EBV+ (UMC-EBV+).

Por último, en el cuarto artículo discutimos las principales características clínicas, histológicas y moleculares de una serie de 13 casos de linfoma B primario cutáneo de la zona marginal (PCMZL) con presencia de más de un 10% de células grandes CD30+ con morfología Hodgkin-like, y analizamos su relación con la progresión clínica e histológica de la enfermedad, así como con otras características inmunofenotípicas.

PALABRAS CLAVE

Linfomas cutáneos, Expresión de CD30, alteraciones moleculares, terapia dirigida, linfoma B primario cutáneo de la zona marginal, úlcera mucocutánea EBV+, linfoma anaplásico de células grandes.

SUMMARY:

CD30 expression in tumor lymphocytes has gained importance since the appearance of Brentuximab Vedotin (BV), a drug that uses this receptor as a therapeutic target and has shown promising results in many patients. The expression of this marker is not uncommon in both B and T cutaneous lymphomas, and it has not been studied in depth for many of them.

This thesis aims to review aspects of the classification of cutaneous lymphomas, which includes clinical, phenotypic and molecular features, focusing on some whose expression of CD30 is significant and poorly studied to date and in which these new phenotypic and molecular characteristics may be relevant in order to achieve better prognostic and therapeutic classification of patients

In the first article we carried out a general review of the most relevant genetic, epigenetic and molecular alterations described in CD30-positive primary cutaneous lymphoproliferative disorders to date, and their possible potential as therapeutic targets.

The second article identifies morphological and immunophenotypic characteristics that help to recognize anaplastic large cell lymphomas with rearrangements of DUSP-22, identifying as common characteristics in these the presence of T-cell markers and the absence of both cytotoxic markers and activation of the JAK / STAT pathway.

In the third article we describe a series of 9 patients with CD30 and EBV positive lymphoproliferative lesions that extend the clinical and histopathological spectrum of EBV + mucocutaneous ulcer (UMC-EBV +).

Finally, in the fourth article we discuss the main clinical, histological and molecular characteristics of a series of 13 cases of primary cutaneous marginal zone B-cell lymphoma (PCMZL) with the presence of more than 10% of large CD30 + cells with Hodgkin-like morphology. We also analyze its relationship with the clinical and histological progression of the disease, as well as with other immunophenotypic characteristics.

KEY WORDS:

Cutaneous lymphomas, CD30 expression, molecular alterations, targeted therapies, primary cutaneous marginal zone B-cell lymphoma, EBV + mucocutaneous ulcer, anaplastic large cell lymphoma.

TABLA DE ABREVIATURAS

LN: Ganglio linfático

LNH: Linfomas No Hodgkin

HL: Linfoma de Hodgkin

PTCLs: Linfomas T periféricos sistémicos

ALCL: Linfoma Anaplásico de Células Grandes

EBV: Epstein Barr virus

EBER: ARN no codificante asociado con el virus de Epstein Barr

HV-like LPD: Enfermedad linfoproliferativa Hydroa vacciniforme-like

CAEBV: Infección crónica activa por virus de Epstein Barr

ARN: Ácido ribonucléico

HIV: Virus de la inmunodeficiencia humana

PEL: Linfoma primario de cavidades

EII: Enfermedad inflamatoria intestinal

PCR: Reacción en cadena de la polimerasa

TCR: Receptor de linfocitos T

IgH: Cadena pesada de las inmunoglobulinas

pcALCL: Linfoma Anaplásico de Células Grandes primario cutáneo

ALCLs: Linfoma Anaplásico de células grandes sistémico

ALCL: Linfoma Anaplásico de células grandes

ALCL ALK+: Linfoma Anaplásico de células grandes ALK-positivo

ALCL ALK-: Linfoma Anaplásico de células grandes ALK-negativo

CTCLs: Linfomas cutáneos primarios

LyP: Papulosis linfomatoide

IPI: Índice Pronóstico Internacional

SS: Síndrome de Sézary

SP: Sangre periférica

MF: Micosis Fungoides

SKHV: Virus herpes asociado a sarcoma de Kaposi

PCMZL: Linfoma B primario cutáneo de la zona marginal

PCFCL: Linfoma B primario cutáneo centrofolicular

DLBCL, EBV+: Linfoma B difuso de células grandes EBV+.

PCDLBLC, LT: Linfoma B difuso de células grandes primario cutáneo, tipo piernas

IVLBCL: Linfoma B difuso de células grandes intravascular

PMN: Células polimorfonucleares

PL: Pitiriasis liquenoide

M: Hombre (Male)

F: Mujer (Female)

OR: Odds Ratio

CI: Intervalo de Confianza

OS: Supervivencia global

PFS: Supervivencia libre de progresión

FDA: Foods and Drugs Administration

CHOP: Ciclofosfamida, adriamicina, vincristina, prednisona

R-CHOP: Rituximab, ciclofosfamida, adriamicina, vincristina, prednisona

CHOEP: Ciclofosfamida, hidroxidanurrobicina, vincristina, etopósido, prednisona

RTX: Rituximab

RT: Radioterapia

SCT: Stem cell transplantation

NED: Sin evidencia de enfermedad

CR: Remisión completa

BV: Brentuximab Vedotin

JAK: Janus Kinase

STAT: Signal transducers and activators of transcription

DUSP22: Dual specificity phosphatase 22 promoter

IRF4: Multiple mieloma oncogene/1 interferon regulatory factor 4

WHO: World Health Organitation

EORTC: European Organisation for Research and Treatment of Cancer

F: Focal

NP: No realizado

W: Débil

A: Ausente

P: Presente

S: Aislada

CMML: Leucemia mielomonocítica crónica

FU: Seguimiento

MDS: Síndrome mielodisplásico

RA: Artritis Reumatoide

ICC: Insuficiencia cardiaca congestiva

CE: Corticoesteroides

N: Nodular

A*: Ausente con folículos desestructurados

D: Difuso

I: Intersticial

M: Mixto

PC: Presente en acúmulos

C: Acúmulos

NE: No evaluable

WT: Wild type

ÍNDICE

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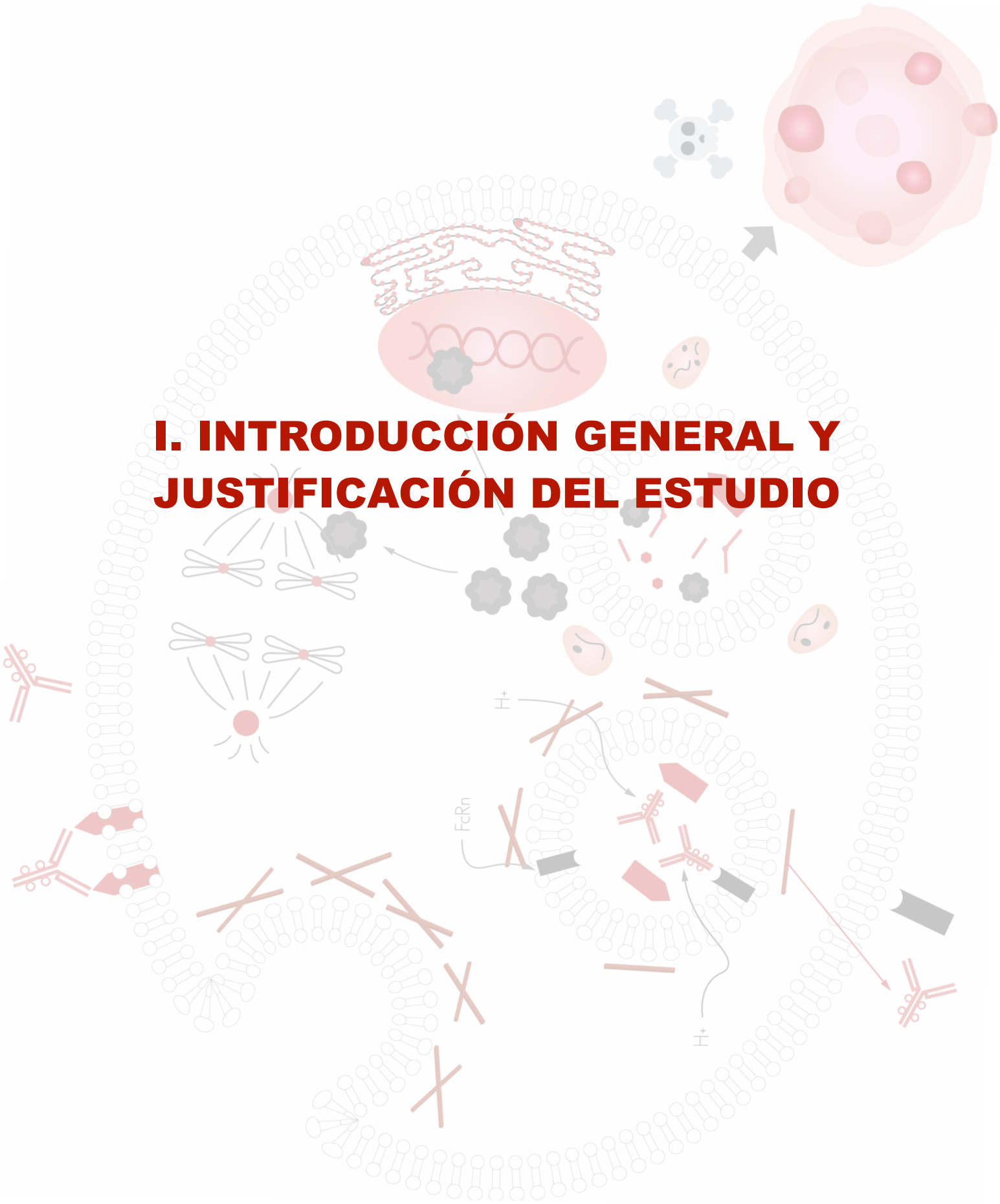
PRIMERA PARTE

I. INTRODUCCIÓN GENERAL Y JUSTIFICACIÓN DEL ESTUDIO



1. LINFOMAS CUTÁNEOS. CONCEPTO Y CLASIFICACIÓN

Los linfomas cutáneos son un grupo heterogéneo de linfomas extranodales que incluyen tanto linfomas B como T, que se presentan primariamente en la piel sin evidencia al inicio de enfermedad ganglionar o sistémica.[1] En la última década el diagnóstico de estas entidades estaba basado en la clasificación de la WHO-EORTC de 2005 actualizada en 2008, pero recientemente, en septiembre de 2018 se ha publicado una nueva versión de esta clasificación en la cuarta edición del libro azul de los tumores de la WHO.[1] Las novedades introducidas recientemente incluyen la inclusión de dos nuevas entidades provisionales, el linfoma primario cutáneo T CD8+ acral y la úlcera mucocutánea EBV+, tratada en esta tesis, al igual que una nueva sección de formas cutáneas de enfermedad crónica activa por EBV.[1] Además la entidad conocida en la anterior revisión de la clasificación como linfoma T CD4+ de células pequeñas y medianas ha sido renombrada como proceso linfoproliferativo, quitándole la denominación de linfoma por su curso clínico indolente. Otras modificaciones introducidas que afectan también al propósito de esta tesis son la ampliación del espectro histopatológico y genético de la papulosis linfomatoide y el reconocimiento de dos subtipos de linfomas primarios cutáneos de la zona marginal.[1] Por tanto, los artículos que componen esta tesis se centran en temas de interés y actualidad revisados en la última clasificación de los linfomas cutáneos.

Para facilitar la compresión de la clasificación inicial y los cambios introducidos recientemente, se incluye un esquema de la clasificación inicial de 2005 de la WHO-EORTC frente a la revisión de 2008 y la revisión actual de la clasificación de 2018. (Tabla 1)

Tabla 1. Evolución de la clasificación de los linfomas cutáneos en los últimos años

WHO-EORTC Classification 2005	WHO Classification 2008	WHO-EORTC Classification 2018
Cutaneous T-cell lymphomas		**Cutaneous T-cell lymphomas**
Mycosis fungoides(MF)	Mycosis fungoides	MF
MF variants		MF variants
Folliculotropic MF		Folliculotropic MF
Pagetoid reticulosis		Pagetoid reticulosis
Granulomatous slack skin		Granulomatous slack skin
Sézary síndrome(SS)	Sézary syndrome	SS
Adult T-cell leukemia/lymphoma	Adult T-cell leukemia/lymphoma	Adult T-cell leukemia/lymphoma
Primary cutaneous CD30+ lymphoproliferative disorders	Primary cutaneous CD30+ lymphoproliferative disorders	Primary cutaneous CD30 LPDs
Lymphomatoid papulosis	Lymphomatoid papulosis	LyP
Primary cutaneous anaplastic large cell lymphoma	Primary cutaneous anaplastic large cell lymphoma	C-ALCL
Subcutaneous paniculitis-like T cell lymphoma	Subcutaneous paniculitis-like T cell lymphoma	Subcutaneous panniculitis-like T-cell lymphoma
Extranodal NK/T-cell lymphoma, nasal type	Extranodal NK/T-cell lymphoma, nasal type	Extranodal NK/T-cell lymphoma, nasal type.
Primary cutaneous peripheral T-cell lymphoma, unspecified		Chronic active EBV infection
	Primary cutaneous CD8+ aggressive epidermotropic cytotoxic T-cell lymphoma	**Primary cutaneous peripheral T-cell lymphoma, rare subtypes**
Primary cutaneous CD8+ aggressive epidermotropic cytotoxic T-cell lymphoma (AECTCL) (*provisional*)	Cutaneous γ/δ T-cell lymphoma	Primary cutaneous γ/δ T-cell lymphoma
Cutaneous γ/δ T-cell lymphoma (*provisional*)	Primary cutaneous CD4+ small/medium-sized pleomorphic T-cell lymphoma	CD8 AECTCL (*provisional*)
Primary cutaneous CD4+ small/medium-sized pleomorphic T-cell lymphoma(*provisional*)		Primary cutaneous CD4+small/medium T-cell lymphoproliferative disorder (*provisional*)
		Primary cutaneous acral CD8+T-cell lymphoma (*provisional*)
		Primary cutaneous peripheral T-cell lymphoma, NOS
Cutaneous B-cell lymphomas		
Primary cutaneous marginal zone B-cell lymphoma (PCMZL)	Extranodal marginal zone lymphoma of mucosa-associated lymphoid tissue (MALT lymhoma)	**Cutaneous B-cell lymphomas**
Primary cutaneous follicle center lymphoma (PCFCL)	Primary cutaneous follicle center lymphoma	PCMZL
Primary cutaneous diffuse large B-cell lymphoma, leg type (PCDLBLC, LT)	Primary cutaneous diffuse large B-cell lymphoma, leg type	PCFCL
Primary cutaneous diffuse large B-cell lymphoma, other		PCDLBLC, LT
Intravascular large B-cell lymphoma	Intravascular large B-cell lymphoma	EBV+ mucocutaneous ulcer (provisional)
		Intravascular large B-cell lymphoma
Precursor hematologic neoplasm		
CD4+/CD56+ hematodermic neoplasm(blastic NK cell lymphoma)	Blastic plasmacytoid dendritic cell cell neoplasm	

2. PRONÓSTICO Y TRATAMIENTO

En general, los linfomas cutáneos tienen mejor pronóstico que sus contrapartidas sistémicas.[2] Sin embargo, como hemos visto previamente, el termino linfoma cutáneo incluye un grupo heterogéneo de entidades, algunas de ellas como el síndrome de Sézary, el linfoma T gamma-delta, los linfomas T periféricos NOS, o los linfomas B difusos de células grandes que tienen un comportamiento más agresivo que otros linfomas cutáneos, que siguen un curso clínico más indolente. Las características morfológicas de las células, incluyendo la presencia de células grandes no está claro que en el caso de los linfomas cutáneos T represente un peor pronóstico, salvo en los casos de MF transformada. Clásicamente la presencia de células CD30 positivas se creía uno de los marcadores más útiles como indicativo de transformación y como consiguiente peor pronóstico en MF, sin embargo, recientemente un estudio postula que la presencia de células p53 positivas y un mayor porcentaje de proliferación demostrado con Ki-67, ayudarían a un mejor diagnóstico de la transformación en la MF.[3]

Los tratamientos utilizados dependen del linfoma en concreto y del estadio en el que se encuentre el paciente e incluyen desde tratamientos locales como la cirugía, los corticoides tópicos o intralesionales, el rituximab intralesional, la fototerapia o la radioterapia, a tratamientos sistémicos como los retinoides orales, el metotrexate, el interferón, fármacos diana como el rituximab iv o el brentuximab y esquemas de quimioterapia convencional como el CHOP.[1]

Clásicamente, el pronóstico de los linfomas, especialmente en el caso de los linfomas sistémicos, se ha basado en la clasificación histológica, el estadiaje y en índices como el IPI que tienen en cuenta características clínicas de los

pacientes. En los últimos años con la llegada de la clasificación molecular de muchos cánceres, incluidos los linfomas, se ha podido observar que determinadas mutaciones confieren mejor o peor pronóstico a los pacientes e implican mejor o peor respuesta a la quimioterapia convencional, a la inmunoterapia o representan dianas terapéuticas sobre las que se puede atacar al tumor con nuevas moléculas. Esto ha resultado especialmente importante en el caso de los ALCLs como trataremos en el último apartado de la introducción de esta tesis.[4]

Los linfomas cutáneos que vamos a tratar en esta tesis tienen en general un curso bastante indolente, y el interés fundamental que despiertan es que en muchas ocasiones su diagnostico diferencial es con linfomas mucho más agresivos que requerirán de tratamientos sistémicos y cuyo pronóstico es mucho más desfavorable. Por ello, creemos que un correcto diagnóstico es fundamental para no sobre-tratar a estos pacientes y que la iatrogenia no tenga consecuencias a corto y largo plazo.

3. PRINCIPALES SUBTIPOS DE LINFOMAS CUTÁNEOS CON EXPRESIÓN DE CD30

El CD30 es un receptor transmembrana que pertenece a la familia de receptores del factor de necrosis tumoral (TNF) con expresión restrictiva en células B y T activadas de tejidos linfoides normales.[5] En su porción intracelular puede unir los factores asociados a receptores TNF TRAF1, 2, 3 Y 5 y estos a su vez pueden activar la vía NFKB.

La expresión de CD30 en linfomas, tanto cutáneos como sistémicos, tiene un interés especial en el tratamiento de los mismos ya que en los últimos años

ha aparecido el Brentuximab Vedotin (BV), un anticuerpo anti-CD30 que combina un agente citotóxico (MMAE) y que ha demostrado buenos resultados en diversos linfomas tanto cutáneos como sistémicos con expresión de CD30.[6] Aunque inicialmente la expresión de esta molécula en más del 10% de las células tumorales era un criterio para su utilización en el tratamiento de muchos tumores, se ha visto que tumores con menos expresión también responden al fármaco.[7] La expresión de CD30 no es exclusiva de una extirpe linfocitaria concreta, por ejemplo, en el caso de linfomas T periféricos (PTCLs) se ha visto que la expresión heterogénea de CD30 ocurre tanto en blastos B como en células B maduras y células T tumorales.[8]

3.1 LINFOMAS T CUTÁNEOS CON EXPRESIÓN DE CD30

A diferencia de lo que ocurre en los linfomas sistémicos, en la piel, los linfomas de células T representan la mayoría de los casos diagnosticados. La presencia de células CD30 se ha descrito en muchos de los linfomas cutáneos de células T. A continuación, realizaré un pequeño resumen de las entidades principales dentro de este grupo en las que la presencia de células CD30+ tiene valor en el diagnóstico y tratamiento de los pacientes.

MICOSIS FUNGOIDE Y SINDROME DE SEZARY

La MF es el linfoma cutáneo más frecuente y representa aproximadamente un 50% de los linfomas primarios cutáneos.[2,9] Fue el primer linfoma cutáneo descrito por el dermatólogo francés Alibert en el año 1806, y clásicamente se divide en 3 fases clínicas: parches, placas y tumores. Aproximadamente un 90% de los pacientes con MF en estadios “tempranos” IA,

IB nunca progresan a estadios tumorales ni presentan afectación extracutánea.[2,9]

El SS es una entidad infrecuente que supone menos del 5% de todos los linfomas cutáneos.[2,9] Se define clínicamente por la triada de eritrodermia, adenopatías generalizadas y la presencia de células neoplásicas T con núcleo cerebriforme en sp, con relación clonal en piel, ganglios linfáticos y sangre periférica. Para su diagnóstico se requiere además uno o mas de los siguientes criterios: un contaje absoluto de células de Sézary $\geq$ 1000/μL en sp, una población expandida de células CD4+ con un cociente CD4:CD8 $\geq$10, y la pérdida de uno o más antígenos T.[2,9]

La MF y el SS son linfomas estrechamente relacionados que se consideran en la clasificación de la WHO como entidades diferentes debido al comportamiento clínico y a la célula de origen.[9] En el SS se cree que las células de origen son células T memoria circulantes (CD27+, CD45RA-, CD45RO+) a diferencia de las células de origen en la MF que se cree que son células memoria CD4+ residentes en la piel.[10]

La presencia de células CD30 positivas en el caso de la MF se ha asociado clásicamente a la transformación de las lesiones y a un pronóstico más agresivo, aunque no siempre es así, habiendo MF transformadas o MF muy agresivas con tumores con menos de 10% de células CD30 o con células medianas y pequeñas.[11] En el síndrome de Sézary tampoco es infrecuente encontrar células tumorales con expresión de CD30.[12] En algunos casos de MF con células grandes con expresión de CD30 es necesario realizar diagnóstico diferencial entre la transformación de la MF y una PL, ya que está descrita la coexistencia de ambas en un mismo paciente y el pronóstico es diferente.[13] Esta

expresión se ha utilizado en ensayos clínicos para valorar la eficacia del BV en estos pacientes.[14-16]

PROCESOS LINFOPROLIFERATIVOS CD30+ PRIMARIOS CUTÁNEOS

En este apartado se incluyen la PyL y el cALCL. Debido a que, el primer artículo de esta tesis hace una revisión exhaustiva de los procesos linfoproliferativos CD30 positivos primarios cutáneos, no voy a extenderme en este apartado de la introducción. (ver artículo 1)

EXPRESIÓN DE CD30 EN OTROS LINFOMAS T CUTÁNEOS

La expresión de CD30 también puede verse en linfomas cutáneos primarios gamma-delta [17] y en linfomas cutáneos extranodales T/NK, tipo nasal EBER positivos[18] o de forma ocasional en HV-like LPD, una forma de CAEBV primaria cutánea.[19] Teniendo esto en cuenta, podemos afirmar que la expresión de CD30 en linfomas cutáneos localizados en panículo adiposo no es de ayuda para diferenciar linfomas más agresivos como los descritos en el párrafo anterior y el pcALCL con afectación paniculítica.

La expresión génica y por inmunohistoquímica de CD30 en 43 linfomas T angioinmunoblásticos y en 8 linfomas T periféricos NOS, incluyendo uno cutáneo, ha sido estudiada por Onaindia et al[8] encontrando que un 90% de los casos expresaban CD30 por inmunohistoquímica en niveles que iban desde un 1 a un 95% de las células incluyendo la expresión en células T tumorales y en células B no neoplásicas.

Estos datos de expresión de CD30 en linfomas T agresivos como los mencionados anteriormente abren una posibilidad terapéutica con BV en estos pacientes.

3.2 LINFOMAS B CUTÁNEOS CON EXPRESIÓN DE CD30

La expresión de CD30 en las células B se relaciona en muchos casos con la activación que estas sufren tras la infección por EBV, es por eso que no es infrecuente encontrar blastos B CD30 positivos en mononucleosis infecciosa y en linfomas B EBV relacionados.[20] Sin embargo, está descrita la presencia de células con expresión de CD30 en linfomas B EBV negativos, tanto cutáneos como sistémicos.[9]

LINFOMA B PRIMARIO CUTÁNEO CENTROFOLICULAR

Kempf W et al describieron en 2014 cuatro casos de linfoma B cutáneo centrofolicular que presentaban expresión difusa de células CD30+.[21] Estos autores postulan que esta entidad debe diferenciarse de otros linfomas B cutáneos con expresión intensa de CD30+. En su serie los pacientes no parecen tener peor pronóstico que otros pacientes con este tipo de linfoma cutáneo, pero el pequeño número de pacientes estudiados es un factor limitante para extrapolar conclusiones.

LINFOMA B CUTÁNEO PRIMARIO DE LA ZONA MARGINAL

La expresión de CD30 en el linfoma B cutáneo primario de la zona marginal no es infrecuente pero hasta el momento está poco estudiada en la

literatura. Este es uno de los puntos que se trata ampliamente en el artículo 4 de esta tesis. (ver artículo 4).

ÚLCERA MUCOCUTÁNEA EBV POSITIVA y LINFOMA B DIFUSO DE CÉLULAS GRANDES EBV+, NOS CUTÁNEO

El espectro de enfermedades linfoproliferativas asociadas a las infecciones virales es amplio y va desde linfadenitis reactivas a linfomas agresivos. Con frecuencia los procesos reactivos y los linfomas indolentes pueden resultar difíciles de diferenciar de los linfomas agresivos. Los herpes virus gamma que incluyen el virus de Epstein Barr EBV y el herpes virus asociado al sarcoma de Kaposi (SKHV) pueden infectar a los linfocitos B y establecer una infección latente que no produce viriones y en la cual únicamente se expresa un número limitado de genes.[22] Las personas que sufren inmunodeficiencias primarias o secundarias tienen un riesgo aumentado de desarrollar enfermedades linfoproliferativas relacionadas con EBV. Además, existen linfomas B sistémicos bien definidos asociados con EBV como el linfoma de Burkitt, el linfoma de Hodgkin clásico o el linfoma plasmablástico.

Estos linfomas tienen un número variable de células B CD20+, CD30+, generalmente de fenotipo activado IRF4-MUM1 positivas con expresión variable de Bcl-6. El pronóstico de estas entidades EBV relacionadas es muy diferente, siendo mejor en la UMC EBV-+ que en la granulomatosis linfomatoide o en el linfoma difuso de células grandes EBV+, NOS cutáneo.

En el tercer artículo de esta tesis discutimos ampliamente las características clínicas, histológicas y de pronóstico de la UMC EBV+, así como

sus principales diferencias con otras entidades EBV relacionadas de mayor agresividad.

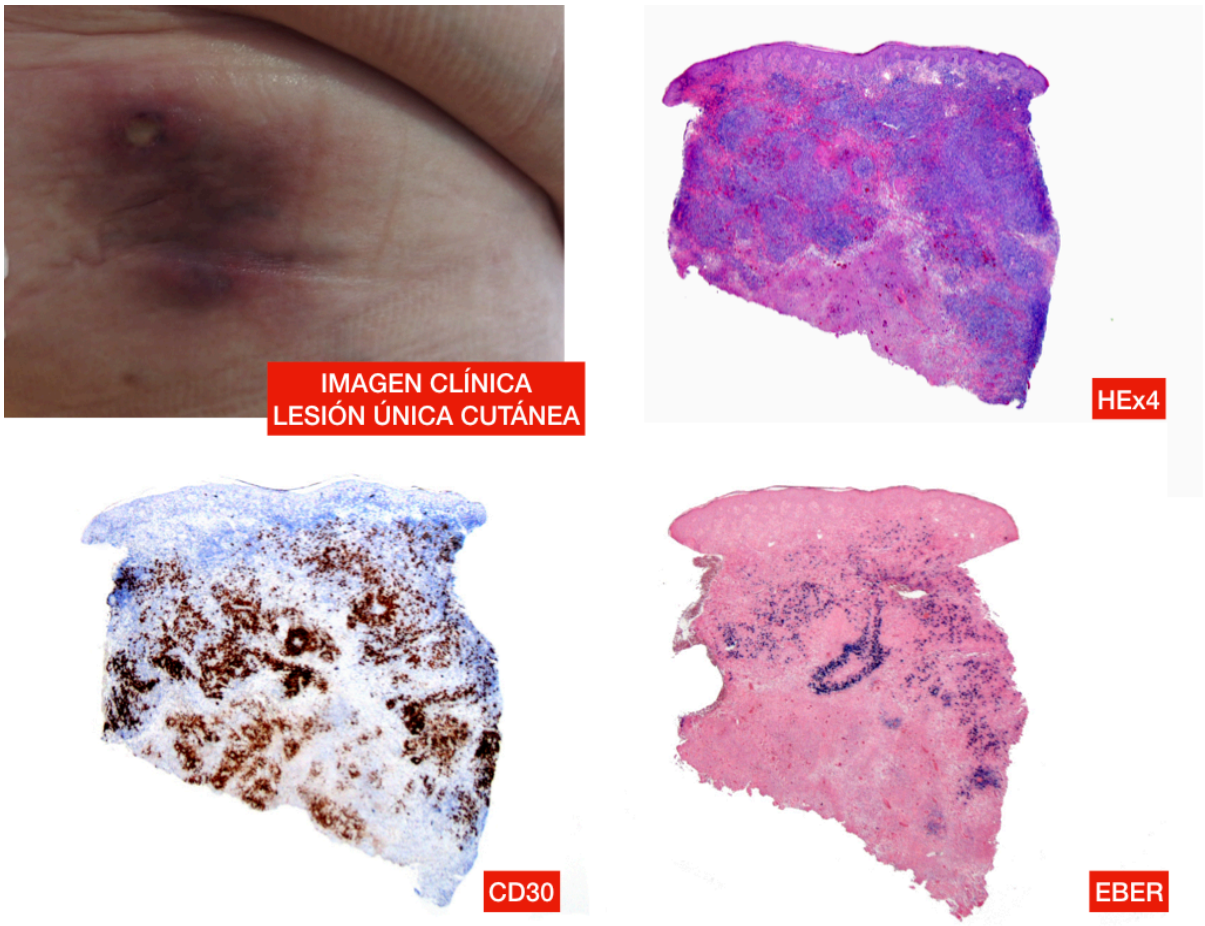


Figura 1. Lesión cutánea única de UMC-EBV+ con patrón difuso y angiocéntrico.

Algunos de estos linfomas EBV-relacionados pueden presentar lesiones cutáneas como ocurre con la granulomatosis linfomatoide, la úlcera mucocutánea EBV+ y el linfoma B difuso de células grandes EBV+, NOS.

LINFOMA B INTRAVASCULAR

Recientemente ha sido publicado un caso de linfoma B de células grandes intravascular (IVLBCL), variante cutánea, con expresión de CD30+.[23]

Este linfoma no debe confundirse con la expresión intralinfática descrita frecuentemente en los procesos linfoproliferativos CD30 positivos (PL, cALCL), en los que la presencia de células CD30+ tumorales en el interior de los vasos linfáticos no implica al parecer un peor pronóstico.[24]

Tabla 2. Adaptada de Cerroni L. *Skin Lymphoma the illustrated guide*. Linfomas cutáneos primarios y secundarios asociados a EBV.

ENTIDAD	FENOTIPO	PATRÓN DE POSITIVIDAD
La mayoría de los casos están asociados a EBV	B	Células grandes neoplásicas Céluas de distinto tamaño
LH clásico	B	Practicamente todas las células
UMC-EBV+	NK/T	Linfocitos angiocéntricos
Linfoma T/NK extranodal, tipo nasal	B	Diferentes % publicados
Granulomatosis linfomatoide	B	Mayoría de células neoplásicas
DLBCL EBV+, NOS	T	Mayoría de las células
Linfoma Hidroa vacciniforme-like	T	Casi todas las células
Hidroa vacciniforme	NK/T	Casi todas las células
Linfoma T/NK intravascular	B	Casi todas las células
Linfoma primario de cavidades (PEL)	B	Nº variable en función del tipo
Linfoma plasmablastico	B	(polimorfo pocas monomorfo muchas)
Enfermedades linfoproliferativas post-transplante y enfermedades linfoproliferativas asociadas a otras deficiencias inmunitarias.	NK	Casi todas las células Células aisladas
Leucemia de células NK agresiva	B(reactivo)y	
Linfoma T angioinmunoblástico	T	
EBV presente en una proporción de casos Linfoma de Burkitt (100% de los endémicos y 15-35% de los Esporádicos)	B	Casi todas las células
EBV asociado en casos esporádicos o anecdóticos publicados en la literatura		
Linfoma T periférico, NOS	T	Algunas células neoplásicas

3.3 EXPRESIÓN DE CD30 EN PSEUDOLINFOMAS CUTÁNEOS

El término “pseudolinfoma” engloba entidades muy diferentes que pueden confundirse clínica o histológicamente con un linfoma cutáneo. Es importante tener en cuenta que “pseudolinfoma” no es un diagnóstico como tal y no debe utilizarse con dicho propósito ya que incluye entidades muy variadas con patogenia y comportamiento clínico totalmente diferente.[2,25]

Los pseudolinfomas cutáneos con expresión de CD30 incluyen una amplia gama de enfermedades cutáneas que histológicamente pueden ser similares a los procesos linfoproliferativos CD30+ primarios cutáneos, en especial con frecuencia a la LyP. Además, cualquier dermatosis reactiva con células linfoides grandes y activadas puede presentar positividad para CD30. Ni siquiera la presencia de agrupaciones de estas células CD30 pueden considerarse diagnósticas de LyP ya que también se han descrito en condiciones reactivas.[26]

Las causas más frecuentes de pseudolinfomas cutáneos CD30 positivos son algunas infecciones, con más frecuencia virales, que incluyen las siguientes: herpes simple, nódulo de Orf, nódulo de los ordeñadores, verrugas virales, molusco contagioso, sífilis, picaduras de insecto, sarna e incluso lesiones cutáneas en infecciones micóticas o en leishmaniasis.[26,27]

Las erupciones medicamentosas linfocitarias también pueden tener numerosas células CD30+ que pueden llegar a simular un linfoma cutáneo primario, pero que normalmente tienen un contexto clínico diferente que ayuda en el diagnóstico diferencial.[28]

En el año 2012 se describió también la presencia de células atípicas CD30 positivas en los infiltrados cutáneos de erupción de recuperación linfocitaria en pacientes con neoplasias, generalmente hematológicas.[29]

En muchos de los casos descritos anteriormente los linfocitos estudiados con técnicas de PCR para los genes TCR gamma y TCR beta resultan policlonales, aunque puede haber excepciones.

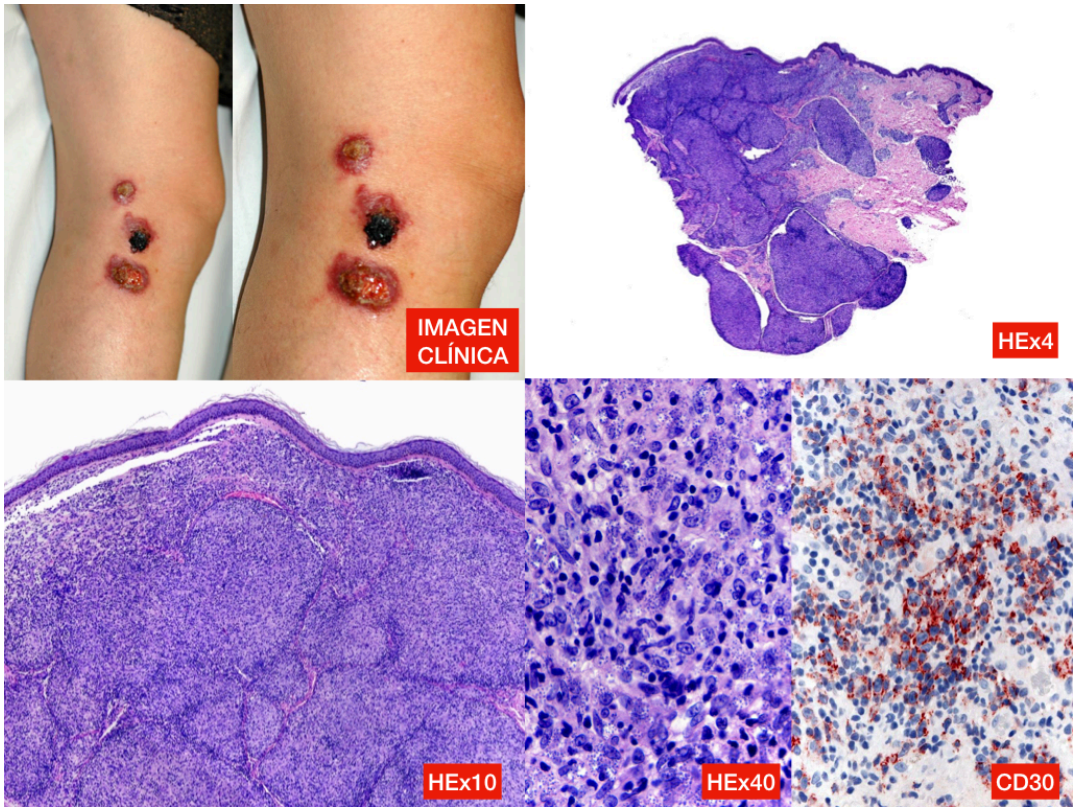


Figura 2. Leishmaniasis cutánea con gran cantidad de células CD30+.

PITYRIASIS LIQUENOIDE (PL)

La pitiriasis liquenoide es una enfermedad cutánea poco frecuente de etiología desconocida. Hay casos de PL en cuya histología hay células CD30+. En los últimos años se ha descrito una forma de pitiriasis liquenoide con rasgos similares a la MF y a la LyP. La evolución clínica de los pacientes es fundamental para realizar el diagnóstico diferencial entre PL y LyP. Parece que la presencia de una población clonal T no es excluyente de PL y no parece que la LyP sea una forma evolutiva en algunos pacientes con PL, sino que se trata de entidades diferenciadas.[30]

4. TERAPIAS DIRIGIDAS EN LINFOMAS CUTÁNEOS CON EXPRESIÓN DE CD30+

Numerosos ensayos clínicos y actualmente la práctica clínica habitual avalan la eficacia de BV tanto en ALCL, HL y otros linfomas T periféricos, incluso en casos refractarios a quimioterapia convencional.[31] En los últimos años han aparecido publicaciones que demuestran la eficacia de BV en linfomas cutáneos, especialmente en MF y SS (ver apartado de MF y SS). Esta utilización como posible diana terapéutica será especificada tanto en el apartado de MF y SS de está introducción, como en el primer artículo que compone la tesis, donde además se tratan otras posibles dianas moleculares en linfomas cutáneos CD30+ que se resumen en la siguiente tabla.

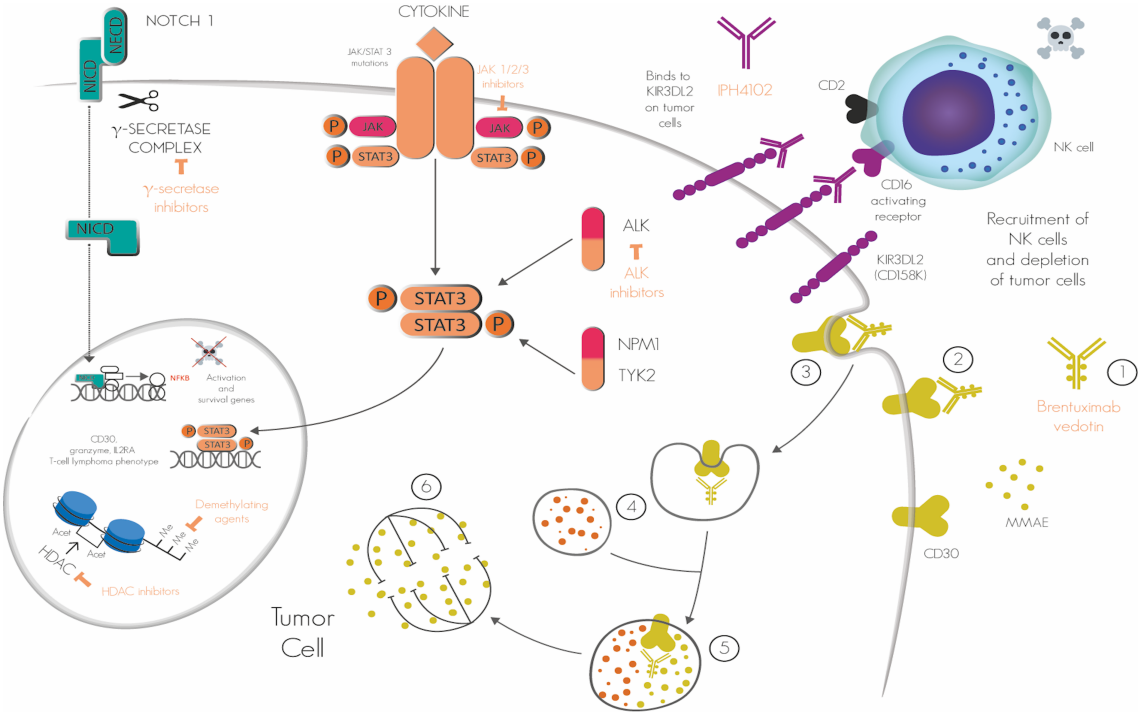


Figura 3. Resumen de posibles dianas terapéuticas en linfomas cutáneos con expresión de CD30. Incluida en artículo 1.

5. LINFOMAS ANAPLÁSICOS DE CÉLULAS GRANDES SISTÉMICOS (ALCLs): CARACTERÍSTICAS CLÍNICAS, INMUNOFENOTÍPICAS Y MOLECULARES.

El linfoma anaplásico de células grandes sistémico (ALCLs) es la entidad más representativa de los PTCLs CD30+ y constituye el tercer tipo más frecuente de PTCLs nodales en nuestro medio. Los ALCLs se clasifican dentro de los linfomas T en la clasificación de la WHO, y tienen un comportamiento biológico muy diferente al de los pcALCL. Se subdividen en ALK+ y ALK- de acuerdo a la presencia de la translocación t(2;5)(p23;q35). Esta translocación afecta con mayor frecuencia a los genes *ALK* y *NPM1*, aunque no siempre, localizados en los cromosomas 2 y 5 respectivamente y conduce a la sobreexpresión de *ALK.*[9]

ALCLs ALK+

Los ALCLs ALK+ constituyen aproximadamente el 3% de los LNH en adultos y el 10-20% de los linfomas en niños y adolescentes.[32] Este linfoma es más frecuente en las tres primeras décadas de la vida y afecta con mayor frecuencia a varones, en una proporción aproximada de 1,5:1.[33,34]

Morfológicamente los linfomas ALCLs ALK+ pueden estar compuestos por células de pequeño tamaño o, por el contrario, predominar las células grandes por lo que se reconocen diversos patrones histológicos.[9] Sin embargo, todos los patrones contienen una proporción variable de células con núcleo excéntrico arriñonado o en herradura, a menudo con una región eosinofílica cerca del núcleo y que se han denominado “células Hallmark”. Algunas de estas células “Hallmark” debido al corte realizado pueden contener pseudoinclusiones que les dan una morfología en “donought”.[9] Respecto al inmunofenotipo, los

ALCLs ALK+ expresan de forma intensa y uniforme CD30 y ALK.[35] La localización subcelular de la tinción de ALK varía en función del gen con el que se reordena ALK, con significación clínica incierta.[36] Aunque la mayoría de ALCLs ALK+ son de origen T, suelen tener un fenotipo T incompleto con pérdida de antígenos pan-T o un fenotipo nulo con pérdida de todos los antígenos T.[33,37] Sin embargo, los marcadores citotóxicos incluyendo perforina, granzima B y TIA1, se expresan con frecuencia, incluso en casos en los que no hay otros marcadores T presentes.[37] Además es común la expresión de EMA, y puede verse positividad para clusterina y CD56.[38,39] Hay casos ocasionales con expresión de marcadores de otros linajes como CD15, Pax5, queratinas o los marcadores mieloides CD33 y CD13.[35]

En cuanto a la genética de estos tumores, los reordenamientos clonales del gen de TCR están presentes en la mayoría de ALCLs ALK+, incluso en los que tienen un fenotipo T nulo.[37] Diversos estudios han demostrado que la fosforilación de STAT3 es necesaria para mantener el fenotipo neoplásico asociado con NPM-ALK.[40,41]. La tirosina cinasa JAK3 parece estar activada de forma constitutiva en líneas celulares de ALCLs ALK+, donde fosforila STAT3 lo que conlleva una regulación al alza de proteínas implicadas en la proliferación y supervivencia celular.[42-44]

La mayoría de los pacientes, un 70% aproximadamente, se diagnostica en un estadio avanzado (III-IV) con afectación periférica y de adenopatías abdominales, frecuentemente con afectación de la médula ósea. Muchos de los pacientes tienen síntomas B que incluyen fiebre alta.[34,45,46] Pese a ser un PTCLs agresivo, el ALCLs ALK+ tiene un pronóstico relativamente bueno con una supervivencia global a los 5 años cercana al 80%, que parece estar relacionada

con que los pacientes con este tipo de linfomas suelen ser pacientes jóvenes que aguantan mejor los régimenes de tratamiento.[4]

ALCLs ALK-

Los ALCLs ALK- son morfológicamente similares a los ALCLs ALK+ con presencia también de células Hallmark y células dounougth, pero carecen de los reordenamientos cromosómicos del gen ALK. A diferencia de los ALK+, la inmunohistoquímica para esta proteína es negativa, sin embargo la tinción para CD30 es fuerte y uniforme, y la tinción tanto de membrana como del Golgi es una característica útil para identificarlos.[35]

Puede verse la pérdida de antígenos T o el fenotipo T nulo, pero es menos frecuente que en los ALK+.[35] Suelen tener un fenotipo citotóxico excepto, como especificaremos en nuestro segundo artículo, los que tienen reordenamientos de DUSP22.

Los estudios realizados demuestran que los ALCLs ALK- son un conjunto de linfomas con gran heterogeneidad clínica y genética y por tanto un pronóstico muy diferente entre sí. [4] Aunque teniendo en cuenta el global de los casos ALK+ y ALK- la supervivencia global de los ALK+ es mejor que la de los ALK-, si se realiza estratificación por las alteraciones genéticas presentes en los linfomas ALK-, estas supervivencias son muy diferentes en función del subtipo genético. En un estudio realizado por Parrilla-Castelar et al con 72 ALCLs ALK- y 32 ALCLs ALK+, identificaron que el 30% de los ALK- tenían reordenamientos de DUSP22 y el 8% de TP63.[4] Los datos de supervivencia global a 5 años eran del 85% en ALCLs ALK+ y del 52% en ALK- de forma global, que estratificando por subtipos genéticos era del 90% en los ALCL ALK- con reordenamiento de DUSP22, del

17% en los ALCLs con reordenamientos de TP63, y del 42% en los casos triple negativos (negativos para los tres reordenamientos ALK, DUSP22 y TP63) (p<0,0001).[4] Los autores destacan que el pronóstico favorable de los pacientes con reordenamientos de DUSP22 parece intrínseco al propio tumor, ya que no podía atribuirse a la utilización de regímenes de quimioterapia más intensivos con trasplante de progenitores hematopoyéticos posterior en estos pacientes, ya que los datos obtenidos eran muy similares en los pacientes no trasplantados.

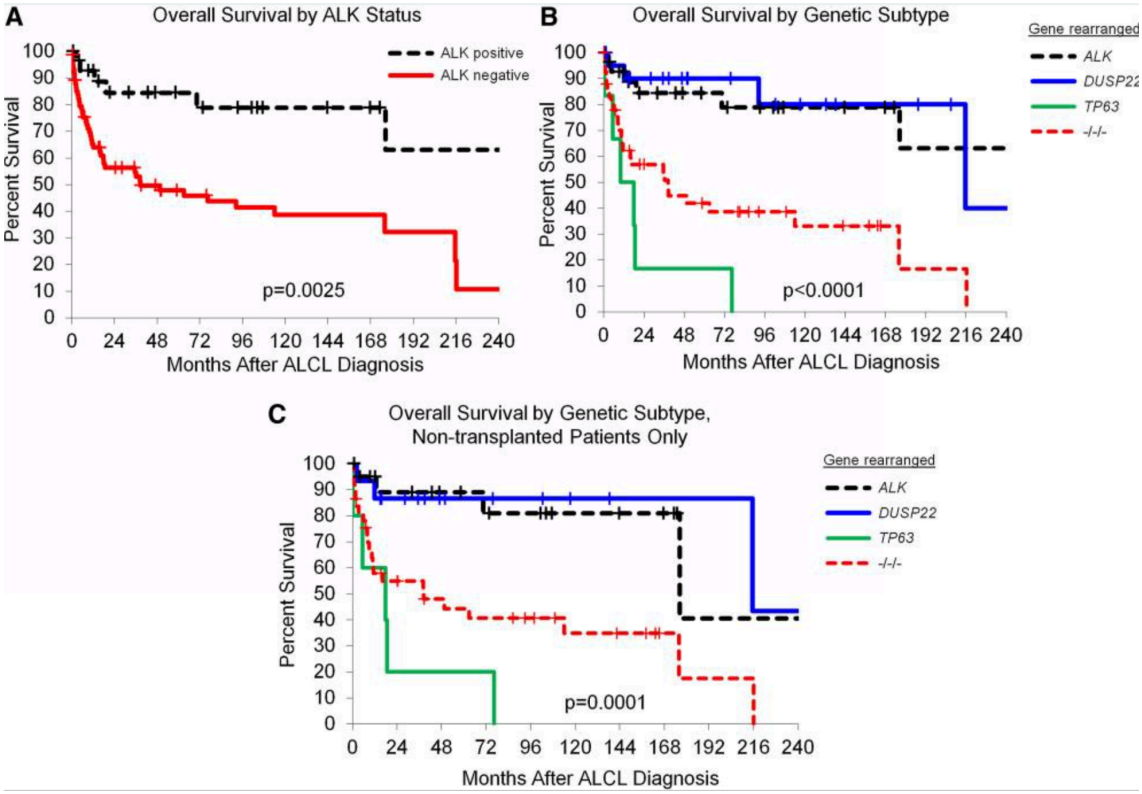


Figura 4. Supervivencias globales de los ALCLs estudiados en el artículo de Parrilla-Castelar et al, por estatus de ALK y por subtipos genéticos en pacientes transplantados y en pacientes no transplantados.[4]

En el artículo 2 de esta tesis hemos estudiado las diferencias morfológicas e inmunofenotípicas que aparecen en los linfomas con reordenamiento de DUSP22, tanto en los cutáneos como en los sistémicos, respecto a otros subtipos moleculares de ALCL.

6. JUSTIFICACIÓN DEL ESTUDIO

Los avances en las técnicas de biología molecular y en el estudio genético y de la biología de los tumores, así como el desarrollo de tratamientos cuya diana de acción son las alteraciones moleculares encontradas, han revolucionado el diagnóstico el pronóstico y el tratamiento de los pacientes con neoplasias y especialmente, con neoplasias hematológicas en los últimos años. Estos avances deben integrarse también en el campo de los linfomas cutáneos y con ello en el trabajo diario de los dermatólogos, patólogos y dermatopatólogos.

Durante años los linfomas cutáneos fueron un cajón de sastre diagnóstico y terapéutico donde muchos pacientes no tenían tratamientos específicos adecuados. Importantes dermatólogos, patólogos y hematólogos han contribuido en los últimos años de forma importante al desarrollo de la clasificación actual de los linfomas cutáneos que implica un mejor diagnóstico, clasificación, pronóstico y tratamiento de los pacientes con esta enfermedad. Sin embargo, aun queda mucho por hacer para conocer mejor la biología de estos tumores, así como poder clasificarlos mejor teniendo en cuenta no solo sus características clínicas e histológicas sino también sus alteraciones moleculares y las características en relación con el pronóstico y de respuesta a las terapias que estas implican. Es por ello que esta tesis nació como un proyecto que integrase las características clínicas, histológicas, inmunofenotípicas y moleculares en el diagnóstico, el pronóstico y el tratamiento de los pacientes con linfomas cutáneos con expresión de CD30.

SEGUNDA PARTE

II. ARTICULOS QUE CONSTITUYEN EL CUERPO DE LA TESIS

La presente tesis es un compendio de trabajos previamente publicados que se centran en cuatro aspectos de interés y actualidad, que se engloban dentro del tema común "Marcadores que permitan mejorar la clasificación de los pacientes con linfomas cutáneos con expresión de CD30 y la selección de terapias adecuadas en función del pronóstico de cada entidad".

Estos cuatro temas, que se tratarán por separado son los siguientes:

1) *Clasificación de los procesos linfoproliferativos T CD30+ primarios cutáneos teniendo en cuenta sus alteraciones moleculares y posibles terapias dirigidas.*
2) *Los linfomas anaplásicos con reordenamientos de DUSP22 tienen características morfológicas específicas y ausencia de marcadores citotóxicos y de la vía JAK/STAT.*
3) *La úlcera mucocutánea EBV-positiva es una entidad que engloba un espectro clínico-histológico de lesiones con buen pronóstico.*
4) *La presencia de células grandes CD30 positivas no es infrecuente en los linfomas primarios cutáneos B de la zona marginal, el aumento del porcentaje de estas células puede acompañar a la progresión de la enfermedad y asociarse con un mayor número de recaídas.*

Artículo 1:

Título: CD30-positive primary cutaneous lymphoproliferative disorders: molecular alterations and targeted therapies.

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Autores: Onaindia A, González de Villambrosía S, Prieto-Torres L, Rodríguez-Pinilla SM, Montes-Moreno S, González-Vela C, Piris MÁ.

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Título: The spectrum of EBV-positive mucocutaneous ulcer: a study of 9 cases.

Autores: Prieto-Torres L, Eraña I, Gil-Redondo R, Gómez de la Riva I, Manso R, Pajares R, Córdoba R, Machan S, Ara M, Requena L, Piris MÁ, Rodríguez-Pinilla SM.

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Título: Large Cells With CD30 Expression and Hodgkin-like Features in Primary Cutaneous Marginal Zone B-Cell Lymphoma: A Study of 13 Cases.

Autores: Prieto-Torres L, Manso R, Cieza-Díaz DE, Jo M, Kilany Pérez L, Montenegro-Damaso T, Eraña I, Lorda M, Suarez Massa D, Machan S, Córdoba R, Ara M, Requena L, Rodriguez-Pinilla SM, Piris MA.

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REVIEW ARTICLE

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CD30-positive primary cutaneous lymphoproliferative disorders: molecular alterations and targeted therapies

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ABSTRACT

Primary cutaneous CD30-positive T-cell lymphoproliferative disorders are the second most common subgroup of cutaneous T-cell lymphomas. They include two clinically different entities with some overlapping features and borderline cases: lymphomatoid papulosis and primary cutaneous anaplastic large cell lymphoma. Molecular studies of primary cutaneous anaplastic large cell lymphoma reveal an increasing level of heterogeneity that is associated with histological and immunophenotypic features of the cases and their response to specific therapies. Here, we review the most significant genetic, epigenetic and molecular alterations described to date in primary cutaneous CD30-positive T-cell lymphoproliferative disorders, and their potential as therapeutic targets.

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Introduction

CD30$^+$ primary cutaneous T-cell lymphoproliferative disorders are the second most common subgroup of cutaneous T-cell lymphomas after mycosis fungoides (MF), accounting for approximately 30% of cases.[1] These cutaneous lymphomas have customarily been classified on the basis of their clinical presentation into lymphomatoid papulosis (LyP), primary cutaneous anaplastic large cell lymphoma (pcALCL) and borderline cases. In recent years, genomic analysis has become important for the diagnosis and clinical management of patients affected by systemic and cutaneous hematologic malignancies.[2] Systemic anaplastic large cell lymphoma (ALCL) is defined by mutually exclusive rearrangements of *ALK*, *DUSP22/IRF4* and *TP63*, which have prognostic and survival implications and must be taken into account in the management of patients.[2] pcALCL have histopathological and immunophenotypic similarities with systemic ALCL, but have a more indolent behavior. Chromosomal translocations described in systemic ALCL can also be seen in pcALCL, although they have different clinical implications. Thus, there are some ALK$^+$ pcALCL, and some cases of LyP and pcALCL share a *DUSP22-IRF4* locus translocation. Bearing all this in mind, we have reviewed the molecular alterations in CD30$^+$ primary cutaneous T-cell lymphoproliferative disorders, describing the various molecular alterations and considering their clinical and therapeutic implications.

Lymphomatoid papulosis

LyP is an enigmatic disease that follows the course of a chronic skin condition and has the histology of a lymphoma. It typically has a recurrent, self-healing course, with an excellent prognosis.[3] Clinical features of all types of LyP are similar and consist of papular, papulonecrotic and/or nodular skin lesions at different stages of evolution. The number of lesions is, however, highly variable, ranging from only a few lesions to hundreds. Likewise, there is great variability in the duration of lesions, which may be present for a few weeks or persist for decades. Lyp

is seen more frequently in adult patients, but children can also be affected.[4]

Customarily, on the basis of its extremely variable histopathology, LyP has been divided into five types with similar prognosis, although distinguishing them is important for the differential diagnosis from more aggressive types of lymphoma.[5] Although more descriptive terms have been proposed, in 2017 the World Health Organization (WHO) categorized LyP using consecutive alphabetical letters.[6] Type A is the most frequent form of LyP, accounting for 80% of cases. Tumor cells are typically $CD4^+$ and $CD30^+$ and appear scattered or in small clusters, accompanied by numerous inflammatory cells, including neutrophils, eosinophils and small lymphocytes. The main differential diagnoses include reactive lesions, such as insect bites, and pityriasis lichenoides et varioliformis acuta (PLEVA).[7] Type B is uncommon, accounting for 5% of cases, and has the same $CD4^+$, $CD8^-$ immunophenotype.[7] It has a histology similar to that of plaque-stage MF with an epidermotropic infiltrate of small, atypical $CD30^+$ cells, which is its main differential diagnosis; less frequently it must be distinguished from cutaneous epidermotropic gamma/delta lymphoma.[5] Type C makes up around 10% of LyP cases and has a histology very similar to that of pcALCL, with a nodular cohesive infiltrate of large $CD30^+$, $CD4^+$, $CD8^-$ pleomorphic and anaplastic tumor cells featuring mitotic figures and abundant cytoplasm.[7] Apart from pcALCL, other entities, such as transformed MF, peripheral T-cell lymphoma not otherwise specified, and adult T-cell lymphoma/leukemia, may have a similar histology.[5] Types D and E have only been described relatively recently, and are usually characterized by a cytotoxic phenotype, with $CD8^+$ and $CD30^+$ lymphocytes. Biopsies from patients with type D LyP show prominent epidermotropism of atypical small-to-medium-sized pleomorphic cells. There may be deep dermal and perivascular infiltrates. This variant accounts for about 5% of cases and needs to be differentiated from pagetoid reticulosis, a peculiar $CD8^+$ form of MF, from more aggressive lymphomas such as primary cutaneous aggressive epidermotropic $CD8^+$ cytotoxic T-cell lymphoma, and from cutaneous gamma/delta lymphoma.[8] Accounting for fewer than 5% of cases, type E LyP shows more extensive necrosis and ulceration due to angiocentric and angiodestructive infiltrates of mostly medium-sized, pleomorphic $CD8^+$ and $CD30^+$ lymphocytes with hemorrhage, vascular occlusion and thrombi, admixed with some eosinophils.[9] Although clinically indolent, the histology can be confused with that of extranodal NK/T-cell lymphoma, nasal type, cutaneous gamma/delta lymphoma or ALCL (primary cutaneous or systemic form) with angiocentric and angiodestructive growth. It is important to highlight that histological differential diagnoses of LyP (such as aggressive epidermotropic $CD8^+$ cytotoxic T-cell lymphoma or MF) must be excluded by clinicopathological correlation based on characteristic clinical grounds with the typical "waxing and waning" presentation of LyP.

Recently, the detection of rearrangements of the *DUSP22-IRF4* locus on chromosome 6p25.3 has enabled the identification of a new molecular-based type of LyP with a characteristic histological pattern.[10]

Lymphomatoid papulosis with 6p25.3 rearrangements

This molecular alteration at the *DUSP22-IRF4* locus is less frequent in LyP than in pcALCL and accounts for fewer than 5% of cases of LyP. Typically, patients are older than those with other forms of LyP, and their lesions are characterized by a biphasic histological pattern showing, on the one hand, extensive epidermotropism with $CD30^+$ small-to-medium-sized T-lymphocytes that simulate pagetoid reticulosis lesions and, on the other, a dermal neoplastic infiltrate composed of large T cells with strong CD30 positivity.[11]

T-cell receptor clonality

Detection of T-cell receptor clonality differs significantly between LyP types.[12] This difference might be related to the number of tumor cells present in the infiltrate, which is lower in type A than in type C, hindering the detection technique in paraffin-embedded tissues. LyP presents atypical evolution for a peripheral T-cell lymphoma, exhibiting self-healing lesions and multiple outbreaks. T-cell receptor clonality studies have been performed in various LyP lesions to investigate the potential role of foreign antigens and the relationship between LyP and other cutaneous T-cell lymphomas in cases with overlapping histological findings. Chott *et al.* showed that multiple skin lesions and associated T-cell lymphomas (MF and ALCL) were clonally related in most LyP patients, which suggests that a non-random genetic event initiates the disease.[13] Shared clonality has been confirmed for LyP and MF lesions occurring in the same patients by de la Garza Bravos *et al.*[14]

T-cell receptor-γ expression is considered a feature of primary cutaneous gamma-delta T-cell lymphoma, and is rare in other types of primary cutaneous lymphoma. However, there are cases of type D LyP with a cytotoxic phenotype and T-cell receptor-γ expression that, unlike in primary cutaneous gamma-delta T-cell lymphoma, is not associated with worse prognosis.[15]

Other findings in lymphomatoid papulosis

SATB1 (special AT-rich sequence-binding protein 1) is an important thymocyte nuclear protein, a chromatin organizer that is crucial to the development of T-lymphocytes.[16,17] SATB1 plays a role in inducing resistance to the death of Sézary cells and has been implicated in the pathogenesis of the leukemic form of cutaneous T-cell lymphoma-Sézary syndrome.[18] Sun *et al.* investigated its expression in a large cohort of patients with $CD30^+$ lymphoproliferative disorders, and studied the potential for its use in classifying $CD30^+$ lymphoproliferative disorders with differential clinicopathological behaviors and prognoses.[19] They identified SATB1 expression in anaplastic T cells in 91.7% of LyP cases and in 38.1% of pcALCL cases. SATB1 cases showed T-helper 17 polarization with expression of T-helper 17 cytokines and repressed T-helper 1-related genes.[19] They also described a better response to methotrexate and interferon treatment in cases with high levels of SATB1 expression. Furthermore, these cases showed more prominent epidermal hyperplasia and granulocytic infiltration.[19] Sun *et al.* postulated that the variability of SATB1 expression in $CD30^+$ lymphoproliferative disorders could be related to the extent of DNA methylation.[19]

Tumor necrosis factor receptor (TNFR)-associated factor 1 (TRAF1) is involved in intracellular signal transduction of a range of TNFR, including CD30 and those associated with nuclear factor-κB activation.[20] Assaf *et al.* studied the expression of TRAF1 using one antibody that recognized

a formalin-resistant epitope (Ber-TRAF1A). They found strong TRAF1 expression in the tumor cells in most LyP cases, in contrast to tumor cells of primary and secondary pcALCL, in which TRAF1 expression was much more restricted.[21] Benner *et al.* also studied this marker in conjunction with MUM1, Bcl2 and CD15, but found it to be of no prognostic or diagnostic utility.[22] We review the role of apoptosis in LyP pathogenesis in greater depth in the section on pcALCL, below.

Mahtas *et al.* studied gene deregulation and spatial genome reorganization near the ALCL translocation breakpoint in ALCL. They described the aberrant expression of Fra2 and of Id2 in LyP, the latter being consistent with the findings of Cotta *et al.*[23,24] They found a lower level of expression in LyP compared with systemic ALCL and argued that gene dosage could be involved in the invasiveness and progression of LyP.[23]

P53 mutations have rarely been found in LyP. Kapur *et al*. reported two cases of LyP with *P53* mutations from an analysis of 11 exons of the *P53* gene. They found *P53* mutations to be infrequent in cutaneous CD30$^+$ lymphoproliferative disorders, and the two patients with LyP who harbored the mutation did not show any changes in the clinical behavior of the disease.[25]

Notch1 expression has been identified in LyP tumor cells, in which it is associated with the expression of the Notch1 ligand Jagged1, but not of the Delta1 ligand, which was expressed at low or negligible levels.[26] The levels of expression of Notch1 and Jagged1 were higher in pcALCL samples, a finding that is discussed in greater detail in the section on pcALCL, below.

To date, no *ALK* fusions have been reported in LyP, in contrast to pcALCL, and no *TP63* rearrangements have been found in these patients.[25,27,28]

Primary cutaneous anaplastic large cell lymphoma

pcALCL is, by definition, a CD30$^+$ large T-cell neoplasm composed of large cells with an anaplastic, pleomorphic or immunoblastic morphology. The CD30 antigen is expressed in more than 75% of tumor cells. pcALCL resembles other forms of ALCL but arises primarily in the skin.[1] The clinical course of pcALCL differs from that of systemic forms of ALCL, both ALK$^+$ and ALK$^-$, which explains why it has been classified as a distinct category.[29] However, there is some overlap between systemic and primary cutaneous forms of ALCL, whereby they share some molecular alterations, suggesting that other genetic and biological differences are likely to exist and therefore need to be identified.[30]

Primary cutaneous large T-cell lymphoma may also be the result of MF tumor progression. Thus, in patients with pcALCL, a current or previous diagnosis of MF must be excluded. The differential diagnosis between pcALCL and transformed CD30$^+$ MF may be challenging, except when there is a clinical presentation with a previous or simultaneous patch-plaque stage MF lesion. Genetic differences between pcALCL and transformed MF have been found using array-based comparative genomic hybridization.[31] From a clinical point of view, patients with pcALCL typically present with solitary or localized nodules or tumors or, more unusually, papules, with frequent ulceration and rapid evolution in some cases that may simulate aggressive lymphomas. The presence of multiple lesions in 20% of cases can hinder the differential diagnosis with type C LyP, which features borderline lesions.[7] This cutaneous lymphoma occurs predominantly in males (male:female ratio 3-2:1). It is more frequent in people in their sixth decade, but may also appear in childhood.[32] It has been reported that pcALCL is a common form of cutaneous lymphoma in immunosuppressed patients, such as individuals infected with human immunodeficiency virus and organ transplant recipients.[33,34] However, in contrast to what usually happens in patients with B-cell lymphomas with CD30$^+$ large cells, especially in immunosuppressed patients, expression of Epstein-Barr virus by the tumor cells is extremely rare or absent in pcALCL with the T/null cell phenotype.[32] When it does appear, it is essential to rule out a diagnosis of a B-cell lymphoma with T-cell markers and CD30 expression, such as plasmablastic lymphoma or primary effusion lymphoma.

There is extracutaneous involvement in about 10% of cases, usually with infiltration of locoregional lymph nodes. In these cases it is important to establish the sequence of presentation in order to rule out cutaneous involvement by systemic ALCL, which has an entirely different prognosis. Characteristically, locoregional lymph node involvement is not related to bad prognosis in pcALCL. Clinical presentation with extensive skin lesions on legs or arms is the only risk factor associated with a statistically significantly worse prognosis.[7,35,36]

The classic histological pattern described in pcALCL consists of a circumscribed nodular infiltrate that is mostly dermal, composed of arranged large lymphoid cells and usually with absent or subtle epidermotropism[5] (Figure 1). However, several variants of this histological pattern have been described, some of which are related to molecular findings described later in this review.[5] Neutrophils and eosinophils are usually scattered in classic forms, although rich variants, which are usually more common in immunosuppressed patients, have been reported. The presence of this rich granulocytic infiltrate may be explained by the release of interleukin-8, whose levels are elevated in cultured tumor cells and in the serum of these patients.[37] Other unusual histological presentations of pcALCL have been reported, including angiocentric or angiodestructive forms,[38,39] subcutaneous and keratoacanthoma-like forms,[40] sarcomatoid variants with prominent spindle-cell morphology,[41] small cell variants that are rarer than in systemic forms of ALK$^+$ ALCL,[42] and an intravascular ALCL that may involve the skin and must be distinguished from the more common intralymphatic spread of tumor cells in pcALCL, which seems to have no prognostic implications.[43,44]

pcALCL cells usually carry an activated T-cell phenotype in which CD30 is expressed in more than 75% of tumor cells (the hallmark of the disease) (Figure 1), frequently accompanied by CD3, CD4 and CD45RO expression and varying degrees of loss of CD5 and CD2. The expression of other activation markers, such as CD71, HLA-DR and CD25 (IL-2R), has been noted in approximately half of the cases. Cytotoxic markers (TIA1, perforin and granzyme B) and cutaneous lymphocyte antigen (CLA, HECA-452) may also be found in around half of the patients.[45] In contrast to systemic ALK$^-$ ALCL, in which approximately 43% of cases are EMA$^+$,[46] EMA is often negative or only focally positive in pcALCL. Interestingly, pcALCL shares with transformed MF and Sézary syndrome the expression of KIR3DL2

(CD158k) by neoplastic cells, a potential target reviewed in the next section.[47]

Altered expression of the T-cell receptor/CD3 complex, T-cell receptor-associated transcription factors and signal transduction molecules is a common feature of systemic and cutaneous CD30+ lymphoproliferations, a finding that may be of potential diagnostic utility.[48]

Monoclonal rearrangement of the *TCR* gene occurs in 65% to 90% of cases of pcALCL in contrast with a much lower frequency in LyP types A and B.[12,49]

Genetic variants of primary cutaneous anaplastic large cell lymphoma

pcALCL seems to carry the same molecular alterations as systemic ALCL, but with dramatic differences in frequencies. From the molecular point of view, most pcALCL cases would currently be classified as triple-negative (*ALK*-, *DUSP22*-, *TP63*-negative), since they do not carry any of these precise underlying molecular alterations. However, these lymphomas have an entirely different prognosis from their systemic counterparts, with some of them showing spontaneous regression and an absence of progression, with long-term survival in the vast majority of the cases (Figure 1). The molecular alterations present in pcALCL are, in descending order of frequency: (i) *DUSP22* rearrangements (Figure 2);[11,50-61] (ii) *ALK* translocations (Figure 3);[62-65] (iii) *TP63* rearrangements;[27,66] and (iv) *NPM1-TYK2* gene fusion.[67-69] *ALK* translocations are much less frequent than in systemic lymphomas, having been described in isolated cases or very small series.[63-65] *TP63* rearrangements and *NPM1-TYK2* gene fusion are exceptional and only isolated cases have been reported.[27,67-69]

ALK-negative primary cutaneous anaplastic large cell lymphoma with DUSP22 rearrangements

DUSP22 rearrangements in systemic ALK- ALCL have been associated with a better prognosis, similar to that of ALK+ systemic ALCL, compared with *TP63*-translocated or triple-negative cases.[50,51] About 20% of pcALCL cases harbor the *DUSP22-IRF4* translocation, with no correlation with MUM1/IRF4 protein expression.[11,52,53] First reported in LyP, a particular biphasic histological pattern has been linked to the presence of *DUSP22* rearrangements in pcALCL (Figure 2). This pattern is characterized

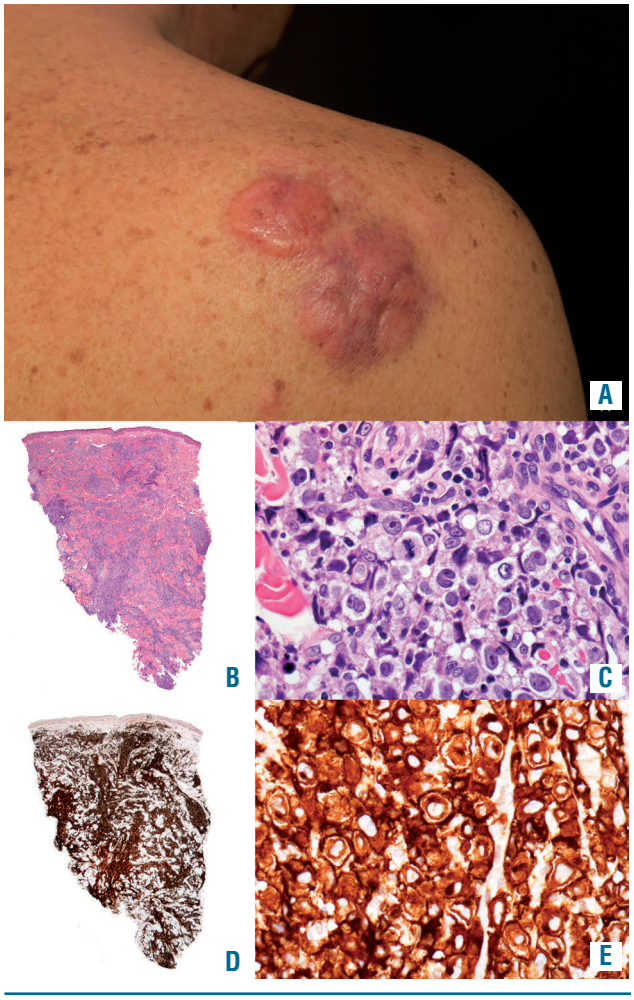

Figure 1. "Classic", *ALK*-, *DUSP22*-, *TP63*- (triple negative) primary cutaneous anaplastic large cell lymphoma. (A) Clinical picture showing two adjacent tumoral erythematous nodules located in the scapular region simulating dermatofibrosarcoma protuberans. (B,C) (x40), Hematoxylin & eosin stain showing the dermal infiltration consisting of a circumscribed infiltrate, composed of arranged large lymphoid cells with absent or subtle epidermotropism. (D,E) (x40), CD30 stain showing positivity in the membrane and Golgi of the tumoral large cells.

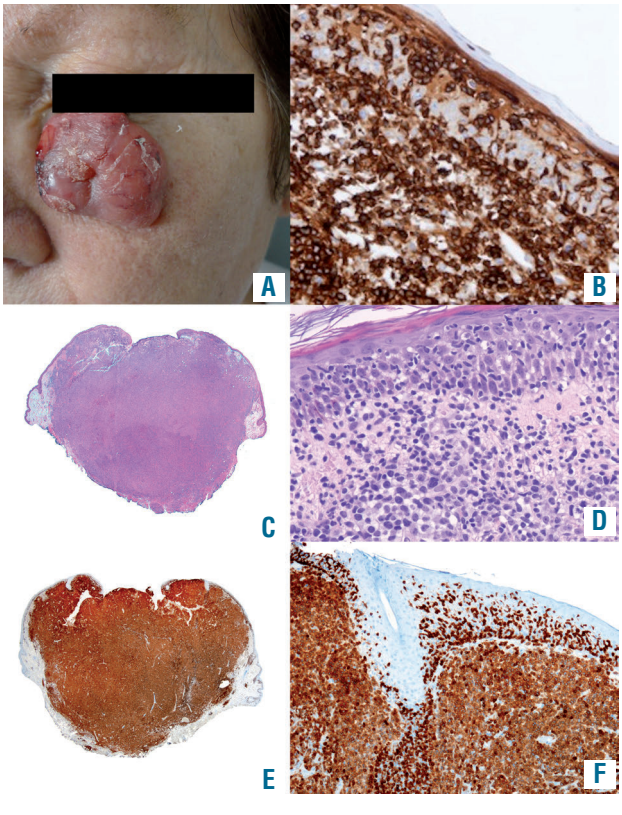

Figure 2. ALK-negative primary cutaneous anaplastic large cell lymphoma with *DUSP22* rearrangements. (A) Clinical picture of a large ulcerated tumor in the left malar region of an elderly woman showing (B) the typical CD30 staining of a case of pcALCL with *DUSP22* rearrangement (Ref. 58, courtesy of Onaindia *et al.*), evidencing extensive epidermotropism with CD30+ small-to-medium-sized T-lymphocytes that simulate pagetoid reticulosis lesions. (C) Hematoxylin & eosin-stained panoramic view of the case in (E) and (F) showing the tumor nodule with profuse dermal involvement. (D) Detail, stained with hematoxylin & eosin, of another case exemplifying the biphasic epidermal and dermal lymphocytic infiltrate. (E,F) (x40), CD3 stain highlights the intraepidermic neoplastic T cells in another case with *DUSP22* rearrangement.

by the simultaneous presence of dermic CD30+ large cells and intraepidermal infiltrate by CD30+ small cells with a pagetoid reticulosis pattern.[10,54] The clinical behavior and prognosis of cases with *DUSP22* rearrangements is similar to that of cases without such rearrangements.[55] Currently, clinical presentation and staging remain essential to differentiate cases of pcALCL with *DUSP22* rearrangement from LyP with this molecular alteration, because the two entities have similar histological features.[54] *DUSP22* rearrangements have also been described in rare cases of CD30-rich tumoral MF.[56]

Expression of the chemokine receptor gene *CCR8* is associated with *DUSP22* rearrangements in ALCL.[57] It has been proposed that the higher level of expression of this skin-homing chemokine receptor may explain the lower tendency of pcALCL to disseminate to extracutaneous sites.[58]

The pathways activated after *DUSP22-IRF4* rearrangements in peripheral T-cell lymphomas have recently been investigated by Mélard *et al.*[59] They found a tumor suppressor function for DUSP22, with the restored expression of DUSP22 promoting apoptosis and impairing soft agar clonogenicity.[59] Negative regulation of the interleukin-6/leukemia inhibitory factor/signal transduction and activator of transcription (STAT)-3 pathway has been found in experimental studies.[60] Consistent with these findings, DUSP22 (also known as JKAP) knockout mice develop inflammation and autoimmunity.[61]

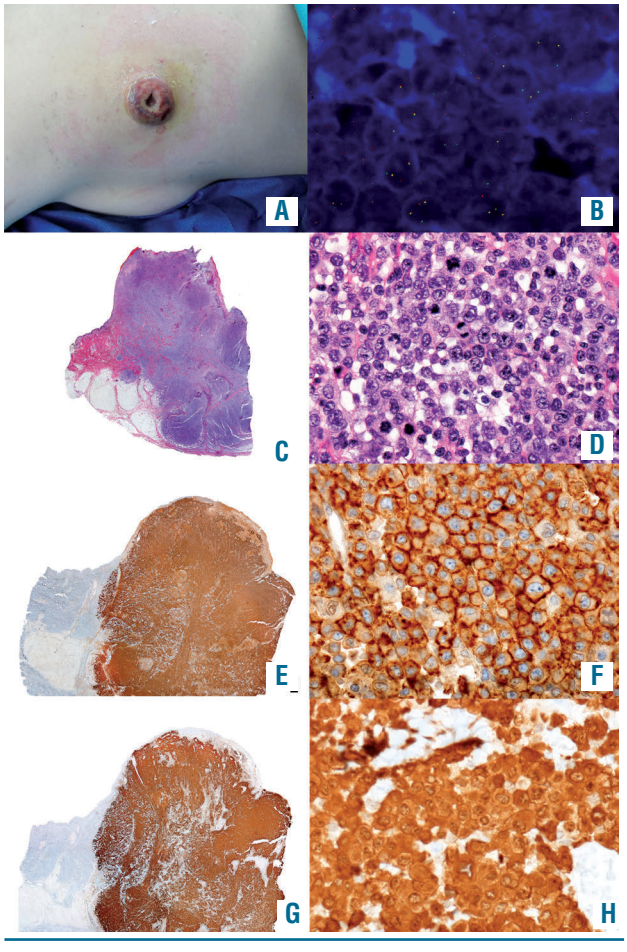

Figure 3. ALK-positive primary cutaneous anaplastic large cell lymphoma. (A) Clinical picture of a large ulcerated tumor located on the back. (B) Fluorescence *in situ* hybridization image showing an *ALK* reciprocal translocation with gain of one or two copies of the *ALK* gene. (C,D) Hematoxylin & eosin stain showing the morphology of large T cells (E,F) (x40), with CD30 positivity and (G,H) (x40), nuclear and cytoplasmic ALK positivity in tumor T cells.

ALK-positive primary cutaneous anaplastic large cell lymphoma

Cutaneous involvement in cases of systemic ALK+ ALCL is generally indicative of a bad prognosis (relative risk: 2) and occurs in 26% of pediatric ALK+ ALCL cases.[62] In contrast, ALK+ pcALCL cases seem to have a more favorable outcome. Only a few cases of ALK+ pcALCL have been reported, the largest series being that described by Oschlies *et al.*,[63] in which six pediatric patients with ALK+ pcALCL had a favorable clinical course, comparable to that of patients with ALK- pcALCL. Other ALK+ primary cutaneous cases occurred in adults and had peculiar features, such as cytoplasmic ALK expression and a lack of *ALK* translocation.[64] To date, no *ALK* fusion partners exclusive to the primary cutaneous cases have been reported. It is important to stage patients with ALK+ pcALCL carefully because cutaneous lesions triggered by insect bites can be the first manifestation of systemic ALK+ ALCL.[65] The authors postulated that insect bite-associated antigens may attract T lymphocytes to the skin, some of which bear the *ALK* translocation t(2;5). The subsequent release of different cytokines at the site of the bite could act as a "second hit" and activate T cells, which may express the oncogenic NPM-ALK protein and initiate deregulated growth.[65]

Primary cutaneous anaplastic large cell lymphoma with TP63 *rearrangements*

In 2012, Vasmatzis *et al.* described two of 19 pcALCL that carried *TP63* rearrangements,[27] both of which had an unusual, aggressive clinical behavior, analogous to that of systemic ALCL with *TP63* rearrangements.[51] Subsequent work by the same group also identified a case of aggressive MF with a *TP63* translocation.[66] In both series, *TP63* translocations were associated with strong TP63 protein expression detectable by immunohistochemistry, but this was not a specific finding since the protein was also expressed in cases without a *TP63* translocation. Schrader *et al.* reviewed a series of 17 cases of aggressive LyP and pcALCL. They found no cases with a *TP63* translocation, and confirmed the lack of specificity of the TP63 immunohistochemistry.[28]

NPM1-TYK2 *gene fusion and oncogenic STAT3 activation*

Mutations on the JAK1/STAT3 pathway are common in systemic ALK- ALCL.[67,68] However, these mutations have been found in only 5% of pcALCL, which is further evidence of the distinct molecular pathogenesis of the two entities. Whole-transcriptome sequencing done in pcALCL revealed the *NPM1-TYK2* gene fusion, which encodes a protein containing an intact catalytic domain in TYK2 and an oligomerization domain of NPM. It was present in two (1 LyP, 1 pcALCL) of the cases of primary cutaneous CD30+ lymphoproliferative disorders studied (n=47) and was not found in other mature T-cell neoplasms (n=151). Both cases showed nuclear STAT5 expression. This defective kinase activates the STAT signaling pathway (STAT1/3/5), implying that TYK2 could be a therapeutic target in this subset of patients.[69]

Epigenetic abnormalities in primary cutaneous anaplastic large cell lymphoma

Epigenetic abnormalities, including histone tail post-translational modifications and DNA methylation, are a

hallmark of cancer and a potential target for therapy.[70] DNA methylation profiling studies have not been done specifically in cutaneous ALCL; data derived from the analysis of systemic ALCL show an ALK-independent methylation signature, characteristic of and different from that of other peripheral T-cell lymphomas, establishing a relation with the promoter DNA methylation of early T-cell stages.[71]

The enhancer of the zeste homolog 2 (EZH2) constitutes the core catalytic subunit of polycomb repressive complex2 (PRC2) and has a "canonical" function as a PRC2-dependent lysine 27 of the histone H3 (H3K27) methylator, which can also methylate a number of non-histone proteins, thereby promoting transcriptional activation, and making it a potential therapeutic target.[72] Yi *et al.* found EZH2 overexpression in pcALCL and large cell transformed cutaneous T-cell lymphomas, in which it regulates apoptosis, cell-cycling in the neoplastic cells, and the interaction between the tumor and its microenvironment.[73]

Non-coding RNA play an important epigenetic regulatory role that has implications for cell development and cancer, above all in the pathogenesis of T-cell lymphomas.[74] MicroRNA (miRNA, miR) are small, non-coding RNA molecules that regulate gene expression at the post-transcriptional level by targeting the 3'-untranslated regions of messenger RNA to promote their degradation or decrease their translation.[75] Several studies have identified different miRNA signatures for CD30+ pcALCL.[62,76-78] Benner *et al.* showed upregulation of an oncogenic miRNA signature in pcALCL comprising miR-155, miR-27b, miR-30c and miR-29.[77] Sandoval's study confirmed the upregulation of miR155 and identified upregulation of miR-21, miR-142-3p/5p, let-7i, miR-424, miR-431, miR-542-5p, miR-29b-1, miR-342-p, and miR-484, with downregulation of some interesting tumor-suppressor miRNA such as miR-23b/miR-27b, miR-203, miR-205 and miR-125b.[78] This miRNA profile reported for pcALCL differs considerably from those reported in systemic ALCL, which suggests a different underlying pathogenic mechanism or reflects differences in the microenvironment[79,80]

In this setting, epigenetic therapy (histone deacetylase inhibitors, such as romidepsin or belinostat, and methylation inhibitors) has shown some benefit in the treatment of ALCL and cutaneous T-cell lymphomas.[81,82] In the future, more precise identification of miRNA profiling in pcALCL could allow us to develop specific diagnostic and progression markers and may lead to the use of more specific targeted therapies.

NOTCH signaling in primary cutaneous anaplastic large cell lymphoma

The deregulation of Notch signaling in hematopoietic cells has been linked to the development of several hematologic malignancies, including acute lymphoblastic T-cell leukemia, B-cell chronic lymphocytic leukemia, multiple myeloma, acute myeloid leukemia, Hodgkin lymphoma and systemic ALCL.[83-88] Increased expression of Notch signaling molecules has been described in primary cutaneous CD30+ lymphoproliferative disorders, as previously mentioned.[26] The same group found that pcALCL cells increased their expression of the intracellular domains of Notch receptors Notch1, Notch2, Notch3 and Notch4, as well as of the Notch ligand Delta and the product HES1.[89] In addition, it was demonstrated that the inhibition of the Notch pathway through inhibition of gamma-secretase with gamma-secretase inhibitors induces apoptosis and decreases cell viability in pcALCL cell lines, findings that identify the Notch pathway as a potential therapeutic target in pcALCL.[89]

CDKN2A-CDKN2B losses in primary cutaneous anaplastic large cell lymphoma

The *CDKN2A-CDKN2B* locus on the 9p21 chromosomal band encodes the proteins p14ARF, p16INK4A and p15INK4B. p14ARF-mdm2-p53 and p16INK4A/p15INK4B-Rb1 pathways are important for controlling the cell cycle, especially progression between G1 and S phases.[90] Combined *CDKN2A-CDKN2B* deletion has been linked to aggressive behavior in cutaneous T-cell lymphomas,[91-95] but more rarely in pcALCL, as confirmed by two research groups.[95,96]

Other cytogenetic abnormalities in primary cutaneous anaplastic large cell lymphoma

Additional chromosomal alterations, such as gains of 7q31 and losses on 6q16-6q21, 6q27 and 13q34 regions, are recurrently present in pcALCL.[31,62,97] Interestingly, these alterations in pcALCL occur mainly in telomeric and centromeric regions. Genomic imbalances have been found in chromosomal regions coding for FGFR1 (8p11), NRAS (1p13.2), MYCN (2p24.1), RAF1 (3p25) and others.[98]

Targeted therapies for primary cutaneous CD30-positive lymphoproliferative disorders

Currently, complete surgical excision and local radiotherapy are the recommended first-line therapies for solitary or grouped localized pcALCL lesions.[99] However, the most appropriate treatment in the relapsed/refractory setting has not been clearly identified. Multiagent chemotherapy is customarily indicated for extracutaneous tumor spread beyond locoregional lymph nodes. In recent years, brentuximab vedotin, an anti-CD30 antibody-drug conjugate has been proposed as one of the best options for achieving complete remission with low toxicity in these patients.[100] ALCANZA, an international, open-label, randomized, phase 3, multicenter clinical trial, has shown significant improvement in objective responses lasting at least 4 months with brentuximab vedotin compared with the physician's choice of methotrexate or bexarotene in CD30+ cutaneous T-cell lymphomas, especially MF and pcALCL (having excluded Sézary syndrome and LyP from the study, since these are entities in which brentuximab vedotin is successful).[101] The mean duration of response to this drug in pcALCL is 7.6 months. Peripheral neuropathy and fatigue are the most commonly reported adverse events (in 57.2% and 35.6% of cases, respectively).[102] Recently the US Food and Drug Administration and the European Medicines Agency approved the use of brentuximab vedotin in cutaneous T-cell lymphomas.

Besides the use of anti-CD30 molecules in the treatment of primary cutaneous CD30+ lymphoproliferative disorders, molecular data generated in the study of pcALCL offer multiple opportunities for targeted therapies (Figure 4).

The recognition of convergent mutations and kinase fusions leading to STAT3 activation in a subgroup of pcALCL could allow the use of JAK1/2/3 inhibitors in the

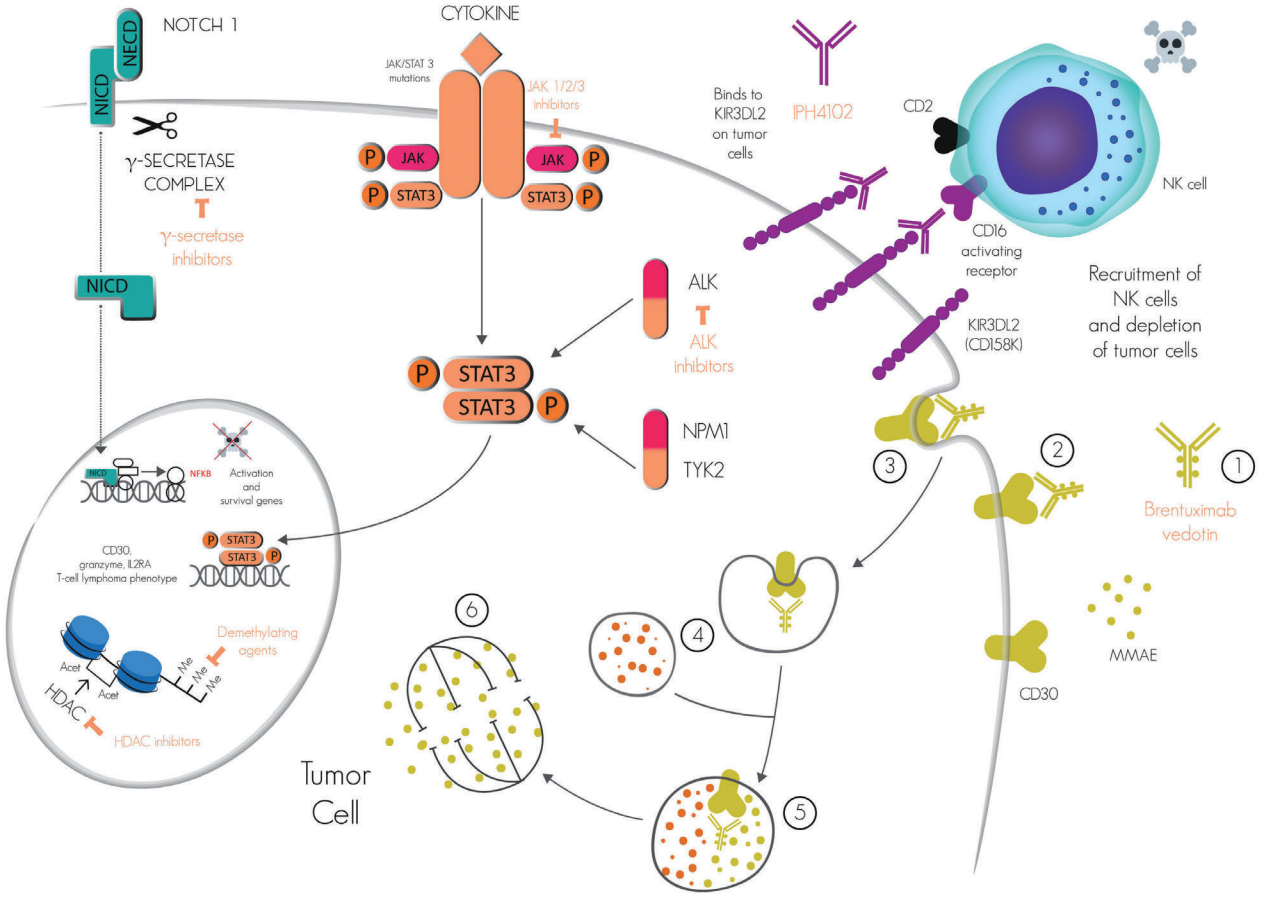

Figure 4. New targeted therapies in advanced primary cutaneous anaplastic large cell lymphoma. Brentuximab vedotin is a drug composed of a chimeric anti-CD30 antibody linked to four monomethyl auristatin (MMAE) molecules (on average) (1). This antibody-drug conjugate first binds to CD30 on the surface of pcALCL cells (2). It is then internalized by a receptor-mediated endocytosis process (3 and 4). The resultant vesicle fuses with lysosomes (4), leading to proteolytic cleavage of the dipeptide linker and the presence of free MMAE molecules, which inhibit tubulin polymerization of the cellular cytoskeleton and arrest growth of pcALCL tumor cells. Gamma-secretase (γ-secretase) inhibitors prevent release of intracellular NOTCH1 (ICN1) from membrane-tethered heterodimeric NOTCH1 protein. This causes the downregulation of the tumor cell nuclear factor-κB (NFKB) pathway and the inactivation of survival genes. JAK1/2/3 inhibitors are effective *in vitro* for controlling pcALCL cell growth. This mechanism involves oncogenic JAK1 or STAT3 mutations associated with the hyperactive pSTAT3 shown in pcALCL with an *NPM1-TYK2* gene fusion and oncogenic STAT3 activation. In addition, anti-ALK molecules such as crizotinib, alectinib, and ceritinib in pcALCL patients with *ALK* rearrangements could downregulate the STAT3 pathway, ultimately inducing tumor-cell death. IPH4102 is a humanized monoclonal antibody directed against the cellular receptor KIR3DL2 (CD158K). This receptor has been shown to be aberrantly expressed in advanced pcALCL. IPH4140 targets KIR3DL2 in tumor cells and promotes cell lysis after linking to the CD16 activating receptor through antibody-dependent, cell-derived cytotoxicity mediated by NK cells. At the epigenetic level, histone deacetylase (HDAC) inhibitors and demethylating agents have demonstrated a degree of effectiveness at inducing cell-cycle arrest, differentiation and/or apoptosis of tumor cells.

treatment of this subgroup.[68,103] In addition, patients with the rare subtype of ALK$^+$ pcALCL could benefit from therapy with the ALK kinase inhibitor crizotinib, and other new-generation inhibitors such as alectinib or ceritinib.[104]

KIR3DL2 (CD158K) is a killer cell immunoglobulin-like receptor normally expressed by minor subsets of circulating CD8$^+$ lymphocytes and natural killer cells.[105] In contrast, tumor cells of pcALCL and CD30$^+$ lymphoproliferative disorders, which are derived from cell lines Mac2a and Mac2b, express KIR3DL2 strongly.[105] In Sézary syndrome, the expression of KIR3DL2 in malignant blood cells has been proposed as a marker of blood tumor burden.[106] The novel agent IPH4102 is a monoclonal antibody directed against KIR3DL2 that has proven effective in *in vitro* cell lines of advanced pcALCL.[47]

The NOTCH pathway is activated in pcALCL as a consequence of various mutations.[89] Deregulated activity of the NOTCH pathway can be inhibited using gamma secretase inhibitors, as has been shown in an *in vitro* experiment.[89]

Finally, therapies acting at the epigenetic level, based on the mutations and epigenetic alterations described above, could be useful in pcALCL. More specifically, romidepsin and vorinostat, inhibitors of histone deacetylase, derived from the bacterium *Chromobacterium violaceum* are effective at inducing apoptosis with an antitumor effect in cutaneous T-cell lymphomas, alone and in combination.[107,108] Recently, researchers from the Yale School of Medicine, using cell lines derived from patients with advanced MF and Sézary syndrome (MyLa, Sez4, HH, Hut78), observed *in vitro* that bromodomain and extraterminal (BET) protein inhibitors are synergistically potentiated by BCL2 inhibitors (e.g., venetoclax) and histone deacetylase inhibitors (e.g., vorinostat and romidepsin) in cutaneous T-cell lymphomas with *MYC* oncogene amplification.[109] Patients with cutaneous CD30$^+$ lymphoproliferative disorders with *MYC*-induced Id2 overexpression could also be candidates for this therapy.[24]

Recently, it was proposed that miR-155 inhibition could be used in combination with apoptotic treatments

and checkpoint inhibitors in MF patients.[110] MiR-155 is also upregulated in pcALCL,[77] providing an additional therapeutic opportunity for these tumors.

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DUSP22-rearranged anaplastic lymphomas are characterized by specific morphological features and a lack of cytotoxic and JAK/STAT surrogate markers

ALK-negative anaplastic large cell lymphoma (ALK-negative ALCL) is a heterogeneous disease with very disparate outcomes. Molecular studies have identified chromosomal rearrangements involving the *DUSP22-IRF4* locus on 6p25.3 (*DUSP22* rearrangements) as a favorable prognostic factor, associated with complete remission after first treatment thereby suggesting that this subgroup of patients may not gain additional benefit from autologous stem cell transplantation in first remission.[1-3] Recognition of these cases is critical, and we therefore aimed to study in greater detail the histological and immunophenotypic features of *DUSP22*-rearranged ALK-negative ALCLs.

After approval by the Institutional Review Board of the Hospital Universitario Marqués de Valdecilla and the Fundación Jiménez Díaz, Spain, we collected 91 cases with a diagnosis of systemic or primary cutaneous ALCL made at the participating institutions. Clinical data were retrieved and cases were reviewed by 3 independent pathologists (AO, SMRP, and MAP) using hematoxylin & eosin stains. Immunohistochemistry was performed using a panel of antibodies against ALK, CD3, CD4, CD8, granzyme B, MUM1, perforin, P-STAT3 (D3A7, 1/400 Cell Signaling), TIA1, P-STAT5, TCR-βF1, P63, STAT3 (*Online Supplementary Appendix*). Of 91 evaluated cases, 18 were primary cutaneous ALCLs (pcALCLs) and 73 cases were systemic ALCLs (19 were ALK-positive ALCLs). ALK-positive cases were not further considered for the study. Only 31 cases were eligible for further study due to tissue scarcity, including 22 ALK-negative ALCL and 9 pcALCLs. Fluorescence *in situ* hybridization (FISH) analyses were performed on these cases using an IRF4-DUSP22 (6p25.3) break-apart probe (KBI-10613; Kreatech, Leica, Spain) following standard procedures.[4,5] Cytotoxic markers, pSTAT3, p63 and MUM1 expression were evaluated as described in the *Online Supplementary Appendix*. Associations of genetic and immunohistochemical subgroups with overall survival (OS) and progression-free survival were assessed using Kaplan-Meier curves. Differences between genetic subgroups in patients' characteristics, tumor phenotype and other clinical factors were assessed using the χ^2 test and Wilcoxon rank-sum test, as appropriate.

Of the 31 cases tested for p63 rearrangements, 1 case (1 out of 31, 3.2%) was positive, 26 were negative (26 out of 31, 83.8%), and 3 showed gains of p63 (3 out of 31, 9.7%). One case (1 out of 22, 4.5%) had *DUSP22* gains, and another case had *DUSP22* amplification. Twenty-five cases (25 out of 31, 80.6%) were classified as triple-negative ALCLs, and 6 cases had *DUSP22* rearrangements, including 4 ALK-negative ALCLs (4 out of 22, 18.2%) and 2 pcALCLs (2 out of 9, 22.2%), representing the study cohort.

Demographic and clinical characteristics of *DUSP22*-rearranged cases are shown in Table 1. The 6 patients were aged 39-65 years at presentation (mean, 56 years), with a predominance of males (2M:1F). In one of the pcALCL cases (case 5), the lesions were restricted to a single body area (the cheek); the site location was not available for case 6. Systemic *DUSP22*-rearranged cases exhibited a high clinical stage at presentation, with low Eastern Cooperative Oncology Group performance status, International Prognostic Index and Prognostic Index for T-cell lymphomas. One patient had bone marrow involvement at diagnosis and high lactate dehydrogenase levels. Two patients received CHOP-based treatment regimens, and another received radiotherapy. All 3 patients achieved complete remission according to the available clinical information. Only the patient receiving radiotherapy as front-line treatment relapsed nine months after initial treatment. None of them underwent stem cell transplantation. After a median follow up of 55 months, all 4 patients with systemic *DUSP22*-rearranged ALCL were alive without disease. Patients with pcALCL were treated by excision, and there was no recurrence or progression during follow up (Table 1). Median follow-up time from diagnosis for systemic ALCL patients who were still alive was 43 months (range, 3-126 months).

Consistent with the results of previous studies, patients with ALK-negative ALCL had a poorer outcome than patients with ALK-positive ALCL [3-year (y) OS: 52%, 95% Confidence Interval (CI): 36-68% *vs.* 80%, 95%CI: 60-100%; log-rank, *P*=0.156]. Patients with systemic *DUSP22*-rearranged ALCL showed better OS rates than the triple-negative ALCL genetic subtype (3-y OS: 100% *vs.* 28%, 96%CI: 4-72%; log-rank, *P*=0.05, for triple-negative patients) and similar to ALK-positive ALCL patients (3-y OS: 80%, 96%CI: 60-100%; log-rank, *P*=0.422) (Figure 1).

As previously described,[6] *DUSP22*-rearranged ALCLs showed unusual histological features that were consis-

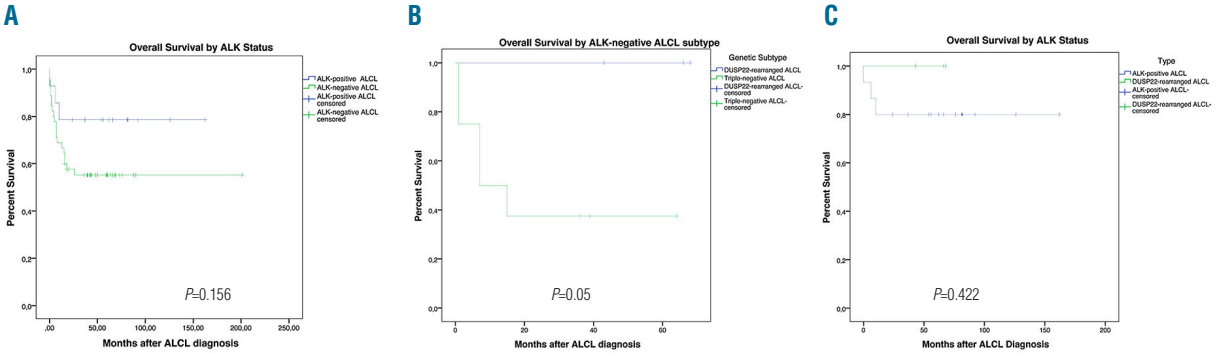

Figure 1. Outcome in patients with anaplastic large cell lymphoma (ALCL) based on genetic subtype. (A) Overall survival (OS) rates in patients with ALCL, stratified by ALK status. (B) OS rates in patients with systemic ALK-negative ALCL, stratified by rearrangements. (C) OS rates in patients with ALK-positive ALCL and DUSP22-rearranged ALCL.

Table 1. Clinical, histological, immunophenotypic, and genetic features of 6 patients with *DUSP22*-rearranged anaplastic large cell lymphoma.

	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6
Clinical presentation						
Age at diagnosis	65	71	50	39	73	39
Gender	M	F	M	M	F	M
Site	Lymph node	Lymph node	Lymph node	Lymph	Skin (right cheek)	Skin
Ann-Arbor Stage	III	IV	–	III	–	–
ECOG Status	0-1	0-1	–	–	–	–
IPI	Low-intermediate	–	–	Low	–	–
PIT	Group 1 (PIT=0)	–	–	–	–	–
Extranodal involvement	Pleural effusion	Skin	–	Tonsil	–	–
Bone marrow involvement	Absent	Present	Absent	Absent	Absent	Absent
Histological features	Hallmark cells	Hallmark cells	Hallmark cells	Hallmark cells	Hallmark cells	Hallmark cells
Cell morphology	Doughnut cells	Doughnut cells	Doughnut cells	Doughnut cells	Doughnut cells	Doughnut cells
Pattern	Sheet-like growth pattern	Sheet-like growth pattern	Sheet-like growth pattern	Sheet-like growth pattern	Biphasic pattern (dermal nodule and pagetoid reticulosis-like epidermal infiltrate)	Biphasic pattern (dermal nodule and pagetoid reticulosis-like epidermal infiltrate)
Background	Inflamatory infiltrate absent. Macrophages with tingible bodies. Apoptotic bodies and mitotic figures.	Inflamatory infiltrate absent. Apoptotic bodies and mitotic figures.	Inflamatory infiltrate absent. Apoptotic bodies and mitotic figures.	Inflamatory infiltrate absent. Apoptotic bodies and mitotic figures.	Inflamatory infiltrate absent. Apoptotic bodies and mitotic figures.	Inflamatory infiltrate absent. Apoptotic bodies and mitotic figures.
Pathological diagnosis	Systemic ALK-negative ALCL	Systemic ALK-negative ALCL	Systemic ALK-negative ALCL	Systemic ALK-negative ALCL	pcALCL	pcALCL
Immunophenotype						
ALK	Negative	Negative	Negative	Negative	Negative	Negative
CD3	Positive	Positive	Positive	Positive	Negative	Positive
CD30	Positive	Positive	Positive	Positive	Positive	Positive
TCR F1	Positive	–	Positive	Positive	Positive	Positive
TIA-1	Negative (10%)	Negative (15%)	Negative (25%)	Negative (5%)	Negative (10%)	Negative (5%)
Granzyme B	Negative (1%)	Negative (0%)	Negative (15%)	Negative (5%)	Negative (1%)	Negative (1%)
Perforin	Negative (1%)	Negative (5%)	Negative (5%)	Negative (0%)	Negative (0%)	Negative (0%)
MUM1	Positive (95%)	Positive (85%)	Positive (100%)	Positive (75%)	Positive (95%)	Negative (0%)
p63	Negative (15%)	Positive (100%)	Positive (85%)	Negative (0%)	–	Negative (0%)
P-STAT1	Negative (<1%)	–	Negative (<1%)	Negative (<1%)	–	Negative (<1%)
P-STAT3	Negative (0%)	–	Negative (15%)	Negative (7%)	Negative (2%)	Negative (10%)
P-STAT5	Negative (0%)	–	Negative (2%)	Negative (2%)	–	Negative (2%)
STAT3	–	Negative (15%)	–	–	–	–
Cytotoxic phenotype	Absent	Absent	Absent	Absent	Absent	Absent
Follow up						
Treatment	CHOP	RT	–	CHOEP	Excision	Excision
Treatment response	CR	CR	CR	CR	–	–
Recurrence/progression	No	Yes (skin)	No	No	No	No
SCT	No	No	No	No	No	No
Status at last follow up	NED	NED	NED	NED	NED	NED
Months since onset	68	66	43	7	1423	12
Months disease free	68	9	43	7	1423	12

ALCL: anaplastic large cell lymphoma; pcALCL: primary cutaneous ALCL; M: male; F: female; CHOP: cyclophosphamide + hydroxydaunorubicin + vincristine prednisone; RT: radiotherapy; CHOEP: cyclophosphamide + hydroxydaunorubicin + vincristine + etoposide + prednisone; RT: radiotherapy; SCT: stem cell transplantation; NED: no evidence of disease; CR: complete remission.

tent among all cases. In the systemic cases, lymph node architecture was effaced, with neoplastic infiltration by intermediate cells that were smaller than those observed in triple-negative and ALK-positive ALCLs, with a sheet-like growth pattern, and a monomorphic appearance. Histopathological findings were consistent among all cases. Neoplastic cells exhibited prominent nucleoli and pseudo-inclusions in the so-called "doughnut" cells, although they were not specific to this group. Hallmark cells, mitotic figures and apoptotic bodies were abundant. Tumor cells were predominant, with no lympho-histiocytic or inflammatory background infiltrate. No sinusoidal involvement was observed, in contrast to the pattern commonly observed in ALK-positive ALCLs (Figure 2). Triple-negative ALCL cases had a more variable morphology, with the presence of hallmark cells and large pleomorphic and multinucleated cells.

The 2 pcALCL cases with *DUSP22* rearrangements had a biphasic pattern, as previously reported by our group.[7] A prominent dermal nodule with a dense lymphoid infiltrate and overlying ulceration was noted at low magnification. The neoplastic infiltrate was composed of medium-to-large atypical cells, with abundant finely granular cytoplasm, intermingled with abundant hallmark cells. A characteristic pagetoid reticulosis-like intraepidermal lymphocytosis pattern was also present, along with intraepidermal small atypical lymphocytes featuring hyperchromatic and irregular nuclei. Mitotic figures and apoptotic bodies were abundant within the dermal infiltrate. Eosinophils and neutrophils were absent (Figure 3).

Among *DUSP22*-rearranged cases, neoplastic cells were positive in all cases for at least one T-cell antigen (Table 1), CD3 and/or the T-cell receptor (TCR) β chain (TCRβF1), negative for ALK, and strongly and diffusely positive for CD30. TCRβF1 stain was not available in case 2, but CD3 was positive. Case 5 was CD3-negative but TCRβF1-positive. These markers accentuated the sheet-like growth pattern in the systemic cases, and the epidermotropic pagetoid reticulosis-like infiltrate in the primary cutaneous cases. All cases had a non-cytotoxic phenotype. TIA-1 was negative in all cases, being found in 5-25% of the tumoral cells. Granzyme B and perforin were also negative in all cases (<5% of tumoral cells). MUM1 was positive in 4 cases (median expression in 95% of tumoral cells, range: 75-100%), and only case 6 was completely negative. P63 expression was more variable, being positive in 2 out of 5 cases tested (85-100% of tumoral cells), and negative (<15% of tumoral cells) in 3

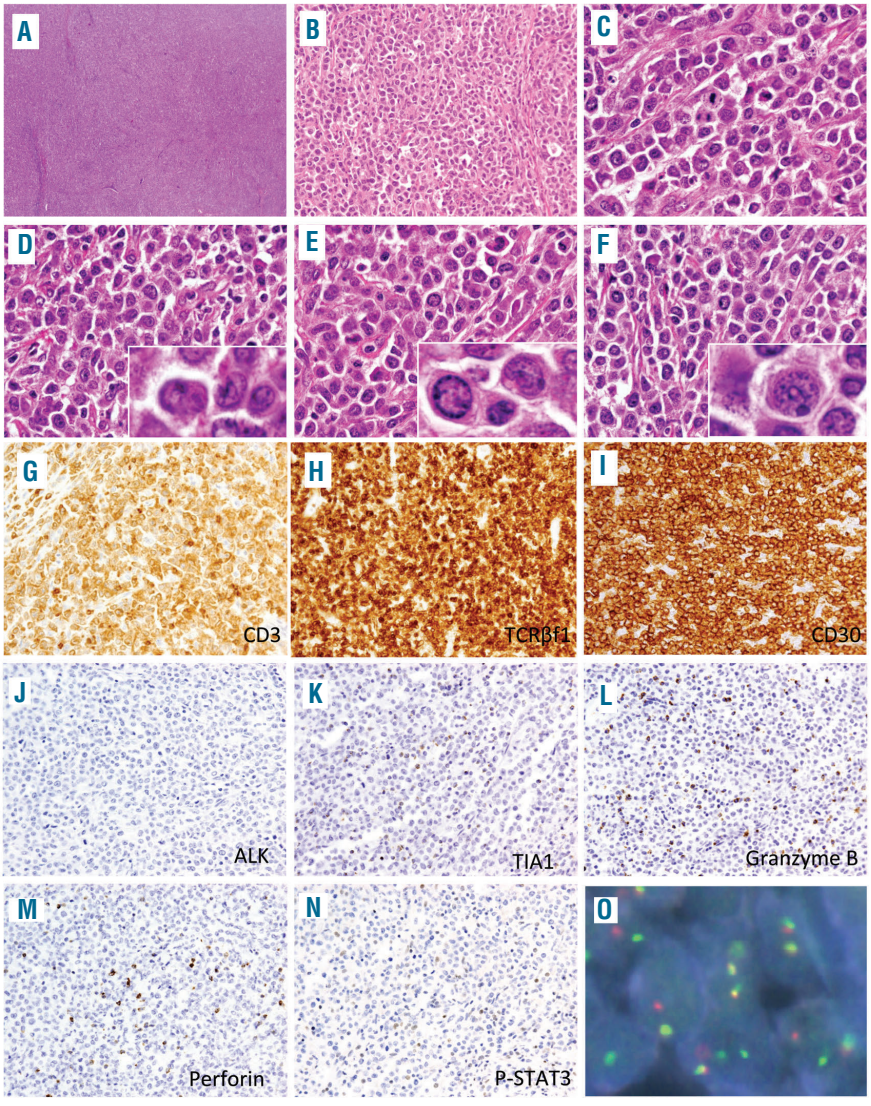

Figure 2. Histological and immunophenotypic features of systemic ALK-negative anaplastic large cell lymphoma with *DUSP22* rearrangement (case 1). (A) Low-power microscopic image of a lymph node with effaced architecture. (B) Sheets of medium-to-large neoplastic cells (C) with abundant hallmark cells, apoptotic cells and doughnut cells (inset), with an eosinophilic nuclear inclusion (D, E and F). Neoplastic cells were diffusely positive for CD3 (G), TCRβF1 (H), and CD30 (I), and negative for ALK (J), cytotoxic markers TIA-1 (K), granzyme B (L) and perforin (M), and for p-STAT3 (N). (O) FISH using a break-apart probe at the *DUSP22* locus shows a rearrangement, with one normal fusion signal and an abnormal split signal.

out of 5 cases. The three surrogate markers of the JAK/STAT pathway (phosphorylated STAT1, STAT3 and STAT5) were consistently negative in all 6 cases (expression in <20% of tumoral cells).

In this study, we report 6 cases of *DUSP22*-rearranged ALCL (systemic and cutaneous) with common histological features, with the presence of intermediate cells with a doughnut-like morphology, and abundant hallmark cells, apoptotic and mitotic figures, as previously reported.[6] In addition, both primary cutaneous cases exhibited a biphasic pattern,[7,8] which has also been described in lymphomatoid papulosis cases carrying the same translocation.[8]

Furthermore, our results support those recently pub-

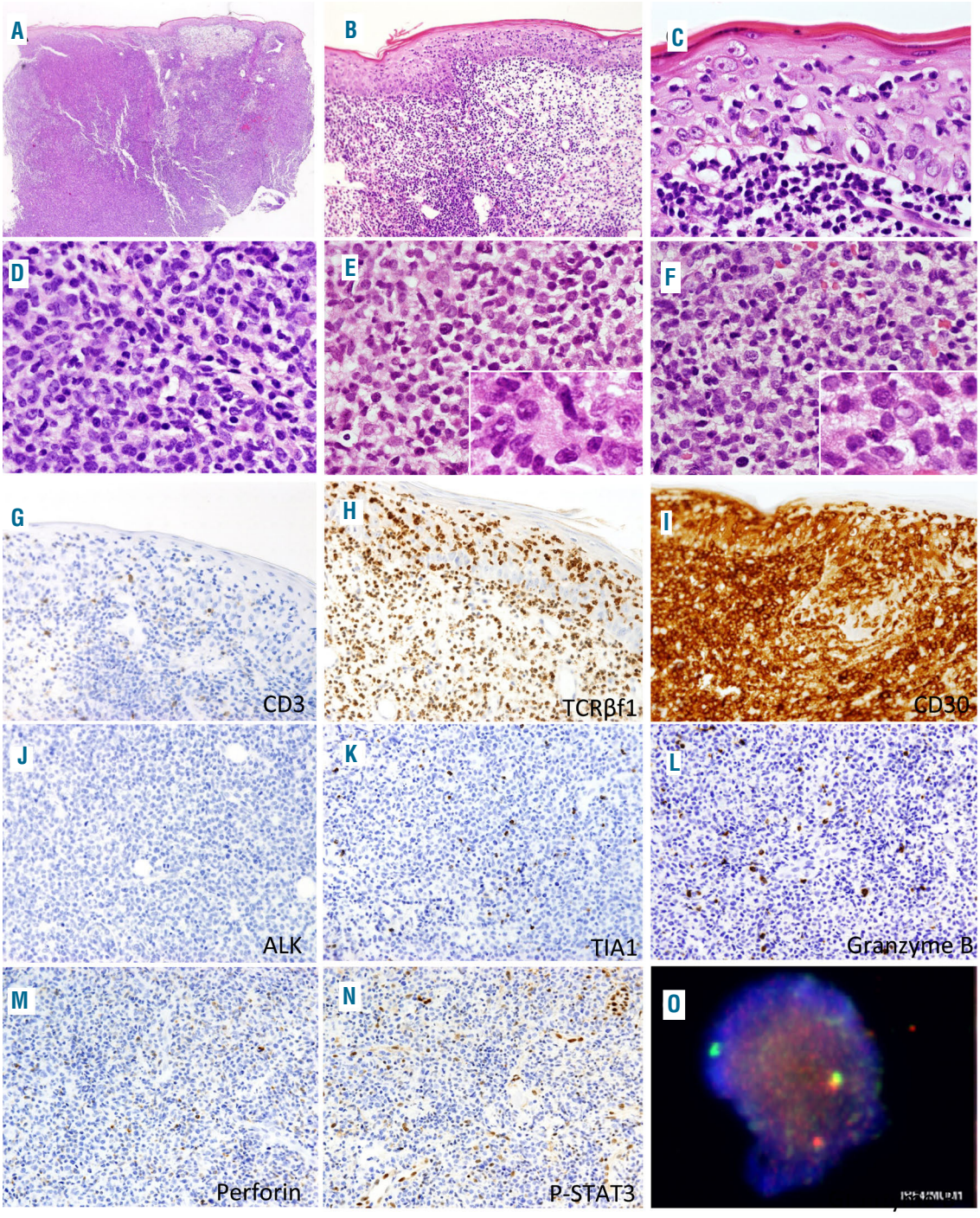

Figure 3. Histological and immunophenotypic features of a primary cutaneous anaplastic large cell lymphoma with *DUSP22* rearrangement (case 5). (A) Low-power microscopic image of the skin biopsy showing diffuse dermal infiltration, characterized histologically by a dense dermal infiltrate with epidermal involvement by small lymphocytes (B and C). (D) Dermal infiltrate of medium-sized and atypical lymphocytes, with a monomorphic appearance, including hallmark and occasional doughnut cells (E, inset; F, inset). Neoplastic cells were CD3-negative (G), TCRβF1-positive (H), and CD30-positive (I). ALK (J), TIA-1 (K), granzyme B (L), and perforin (M), and P-STAT3 (N) were negative. (O) Fluorescence *in situ* hybridization using a break-apart probe at the *DUSP22* locus shows a rearrangement, with one normal fusion signal and an abnormal split signal.

lished by other groups,[9] identifying lack of activation of the JAK/STAT pathway in *DUSP22*-rearranged cases, despite the fact that this had initially been proposed as a universal finding in ALK-positive and ALK-negative ALCLs.[10]

We describe histological and immunophenotypic features that may help recognize *DUSP22*-rearranged cases. The presence of sheets of intermediate-to-large cells, with relatively monomorphous large-cell cytology, including hallmark and doughnut cytology, with no expression of cytotoxic markers, is useful for further FISH testing in systemic cases. In the pcALCL cases, the presence of the previously described biphasic pattern is a useful indicator of *DUSP22*-rearrangement. The same translocation involving locus 6p25 was also described in lymphomatoid papulosis (LyP),[8,11] suggesting that this molecular alteration could determine a better outcome, both in cutaneous and systemic ALK-negative ALCL.

Constant expression of T-cell markers and a lack of cytotoxic markers and markers of activation of the STAT pathways seem to be linked to *DUSP22* translocation in this series.

It would be of interest to explore whether this combination of markers in other ALK-negative ALCLs identifies cases with specific morphology, immunophenotype or clinical features.

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The Spectrum of EBV-Positive Mucocutaneous Ulcer
A Study of 9 Cases

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Abstract: We describe a series of 9 patients with Epstein-Barr virus (EBV)-positive mucocutaneous lymphoproliferative lesions that broadens the concept of EBV-positive mucocutaneous ulcer. We report 5 female and 4 male patients, with an average age of 74 years (range, 55 to 87 y), 2 of whom were HIV-positive. The lesions were located in the oropharynx, skin, and rectal and/or genital mucosa. Histopathologically, 6 cases showed a polymorphic pattern and 3 had a monomorphic and diffuse one, with angiotropism in 4 cases (2 each with the polymorphic and monomorphic patterns). Three of the cases expressed PDL1. In addition to its presence in the neoplastic lymphoid cells, EBV was also detected in adjacent epithelial cells in an oropharyngeal lesion. All cases responded to local therapy or adapted systemic chemotherapy in selected cases. This series extends the spectrum of this disorder to include some HIV-positive cases, patients with multiple lesions confined to a single anatomic area, lesions with an angiocentric pattern, and some cases with monomorphous large-cell cytology. We discuss the differential clinicopathologic diagnosis of this disorder and that of classic EBV large B-cell lymphoma.

Key Words: mucocutaneous ulcer, EBV, immunosuppression, genital lesions, lymphoproliferative disorders

(*Am J Surg Pathol* 2019;43:201–210)

Epstein-Barr virus (EBV)-positive mucocutaneous ulcer (EBVMCU) has been included as a provisional entity in the group of mature B-cell neoplasms in the current 2016 World Health Organization (WHO) Classification of Lymphoproliferative Disorders.[1] Since it was first described in 2010 by Dojcinov et al,[2] this disorder has been a "hot topic" in the hematopathology literature, and about 90 cases have been reported to date in 26 papers.[2–27] EBVMCU was initially described as isolated, sharply circumscribed ulcers located mostly in the oropharyngeal mucosa (16 cases), but also in the skin (6 cases) and gastrointestinal tract (4 cases). All patients described in the original series were receiving immunosuppressive medication or had age-related immunosenescence, and had a median age of 77 years.[2] The lesions were histopathologically characterized by a polymorphous infiltrate with atypical B-cell blasts with a Hodgkin/Reed-Sternberg cell-like appearance.[2] The immunophenotype of the tumoral B cells consisted of CD30 and EBER positivity, with reduced expression of CD20. Characteristically, a background of T cells was also present in the lesions.[2] Clonality studies yielded variable results, with slightly more than one-third of the cases showing clonal Ig rearrangement, a similar proportion with clonal T-cell rearrangement and approximately one-third with a restricted T-cell pattern.[2] Complete remission was achieved in all patients after different treatments, including standard chemotherapy, radiotherapy and cessation of the immunosuppressive drugs cases associated with these medications. Some patients showed spontaneous remission of the lesions.[2] All these data supported the indolent course and good prognosis of the disorder.

Routine hematopathology diagnostic practice has often been challenged by the relatively frequent presence of polymorphic lymphoproliferative lesions in various groups of patients, including those who are HIV-positive, some of whom require a differential diagnosis with large B-cell lymphomas. In this paper, we describe cases of an extranodal EBV-positive lymphoproliferative disorder, with solitary or multiple lesions, but confined to a single anatomic region. The clinicopathologic and immunophenotypic spectrum of the disorder turns out to be wider than initially recognized.

MATERIALS AND METHODS

Case Selection

Cases were identified in the electronic databases of the Pathology Departments of the Fundación Jimenez Diaz

From the Departments of *Dermatology; §Pathology; ‖Hematology, Fundación Jiménez Díaz University Hospital; #Department of Pathology, Fundación Jiménez Díaz University Hospital and CIBERONC, Madrid; Departments of †Pathology; ‡Dermatology, Guadalajara University Hospital, Guadalajara; and ¶Dermatology Hospital, Lozano Blesa Clinical University Hospital, Zaragoza, Spain.

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TABLE 1. Immunohistochemistry: Primary Antibodies Used

Antibody	Clone	Manufacturer	Reference	Control	Antigen Retrival	Amplification	Dilution/ Incubation Time	Stain	Temperature (°C)
CD30-L	Ber-H2	DAKO	IR602	Tonsil	Low	Flex+	RTU 4′	OMNIS	4
CD20cy	L26	DAKO	GA604	Tonsil	High	Flex	RTU 20	OMNIS	4
CD79	JBC117	DAKO	IR621	Tonsil	Low	Flex	RTU 8	OMNIS	4
BCL2	124	DAKO	IR614	Tonsil	High	Flex+	RTU 20	OMNIS	4
CKAE1-AE3	AE1/AE3	DAKO	GA053	Tonsil	High	Flex	RTU 10	OMNIS	4
PAX5	DAK-Pax5	DAKO	IR650	Tonsil	High	Flex+	RTU 15′	OMNIS	4
MUM1	MUM1p	DAKO	IR644	Tonsil	High	Flex+	RTU 30′	OMNIS	4
CD15	Carb-3	DAKO	GA062	Tonsil	High	Flex	RTU 15	OMNIS	4
CD3-L	Polyclonal	DAKO	GA503	Tonsil	Low	Flex+	RTU 10	OMNIS	4
Ki67-L	MIB-1	DAKO	GA506	Tonsil	Low	Flex	RTU 20	OMNIS	4
PD1	NAT105C	CNIO		Tonsil	Low	Flex	1/500 20′	OMNIS	20
PD-L1(28.8)	28.8	DAKO	SK005	Tonsil				AUTOSTAINER Link 48	4
HISTOSONDA EBER	DNP probe	VENTANA	760-1209	EBER+	Protease	Iviewblue ISH	RTU 28′	BENCHMARK	4

RTU indicates ready to use.

University Hospital of Madrid (Spain) and the University Hospital of Guadalajara (Spain). We retrospectively reviewed all the cases, including biopsies performed in the Fundación Jimenez Diaz in Madrid, the University Hospital of Guadalajara, and cases seen in consultations between January 2013 and January 2018. In order to select the cases, we employed a search strategy that included the keywords "EBER ISH," "EBV-POSITIVE MUCOCUTANEOUS ULCER," "EBVDLBCL OF THE ELDERLY," "POST-TRANSPLANT LYMPHOPROLIFERATIVE DISORDER (PTLD)", EPSTEIN-BARR VIRUS," and "EBV-POSITIVE DIFFUSE LARGE B CELL LYMPHOMA (EBVDLBCL)." All cases with disseminated or nodal disease were excluded. At all times, the diagnosis of EBVMCU was guided by the clinical and morphologic characteristics that define the entity according to the most recent (2017) WHO classification. Additional information collected from patients included age, sex, location of lesions, clinical symptoms at diagnosis, clinical morphology of cutaneous-mucosal lesions, personal history of immunosuppression and cause, treatment received, evolution and presence of serological markers of EBV infection (Table 2).

Immunohistochemistry and In Situ Hybridization

The biopsy material used for histopathologic examination was formalin-fixed paraffin-embedded tissue. Paraffin sections of 3 to 4-μm thickness were stained with H&E. A panel of antibodies including CD30, CD20, CD79a, Bcl2, AE1/AE3, Pax5, MUM1, CD15, Ki67, CD3, PDL1, and PD1 was used for the diagnosis (Table 1).

Polymerase Chain Reaction for IgH and TCR Gene Rearrangement

Polymerase chain reaction (PCR) was performed in 3 of our cases (cases 1, 8, and 9) to study the clonal expansion of B cells and T cells. DNA was extracted from paraffin sections and B/T-cell clonal expansion was detected by analyzing the TCRγ gene rearrangement, and PCR for the immunoglobulin heavy (IgH; V_H-J_H and D_H-J_H) chain gene rearrangement.[28] Appropriate positive and negative controls were included in all studies. Clonality was assayed following well-established recommendations.[28]

RESULTS

Clinical Presentation

Nine patients with EBVMCU were identified in our databases (Table 2). There was a slight predominance of women, who made up 55% of the series. The median age at presentation was 74 years (range, 55 to 87 y). Two patients were HIV-positive, well-controlled with antiretroviral treatment, 2 patients were receiving immunosuppressive therapy with methotrexate for rheumatoid arthritis, and one had inflammatory bowel disease treated with mesalazine. One patient had a history of chronic myelomonocytic leukemia, which had been treated in another center 20 years before, and 3 of the older patients had no other previous history of immunosuppression apart from their advanced age.

The clinical lesions of our patients had a variable morphology, covering the entire spectrum described in EBVMCU, with isolated (cases 6 and 7) and multiple (cases 1 and 4) slowly growing indurated ulcers, involving oropharyngeal mucosa (cases 1, 4, 6, and 7) or rectal mucosa (case 3), and ulcers and tumors in the skin (cases 5 and 8). Two patients had previously undescribed clinical presentations including nonspecific erythema of the rectal mucosa, without ulceration or erosion (case 2), and multiple papules and erosions on the glans penis (case 9, Fig. 1A).

In those cases with multiple lesions (cases 1, 4, 6, and 9), all lesions were confined to a single anatomic region (mouth, oropharynx or genital area). There was no hepatic, splenic or bone marrow involvement in any of the cases and the only symptoms were those caused by the local lesions.

Clinical diagnoses were variable, including squamous cell carcinoma for the single oropharyngeal lesions (cases 6 and 7) and a lymphoproliferative process for the HIV-positive

TABLE 2. Clinical Characteristics and Follow-up of Patients in Our Series

Case Number	Age (y)/Sex	Lesion Site	Clinical Presentation	Clinical Morphology	Type of Immunosuppression	Treatment and Outcome	EBV DNA Quantification
1	55/M	Tonsil, cavum, oropharynx, hypopharynx	Severe oropharyngeal bleeding	Multiple ulcers	HIV	Resolution with 4 CHOP cycles FU: 6 mo	Low (84 copies/mL)
2	57/F	Lower rectum	Rectal bleeding	Nonspecific erythema without ulcers or erosions	Inflammatory bowel disease	No rectal bleeding She had the same lesions FU: 9 mo	NP
3	70/M	Rectum	Rectal bleeding	Tumoral lesion	HIV	Spontaneous resolution FU: 9 mo	NP
4	74/M	Left maxillary tuberosity. Palate Left lingual tonsil	Multiple oral lesions	2 ulcers	Methotrexate (RA)	Resolution when drug withdrawn FU: 9 mo	NP
5	87/F	Thigh	Asymptomatic and multiple cutaneous lesions	Violaceous cutaneous nodules	MDS type MMCL	Death due to congestive heart failure	NP
6	75/F	Left mandibular region, gingival	Painful oral lesion	Ulcerated tumor	Oral corticosteroids. Methotrexate (RA)	Resolution when drug withdrawn FU: 66 mo	NP
7	87/F	Floor of the mouth	Painful oral lesion	Ulcerated tumor	Senescence	Resolution after RT FU: 81 mo	NP
8	87/F	Skin in hip zone	Asymptomatic Cutaneous lesion	Infiltrated violaceous plaque	Senescence	Resolution after treatment with bendamustine and rituximab FU: 24 mo	NP
9	74/M	Glans and foreskin	Multiple outbreaks of lesions	Multiple erosive lesions and erythematous papules	Senescence	Spontaneous Resolution FU: 9 mo	NP

CMML indicates chronic myelomonocytic leukemia; F, female; FU, follow-up; M, male; MDS, myelodysplastic síndrome; NP, not performed; RA, rheumatoid arthritis; RT, radiotherapy.

patient (case 1) and the patient receiving methotrexate (case 4). The HIV-positive patient with a rectal tumoral lesion (case 3) was biopsied with suspicion of adenocarcinoma of the rectum. The other patient with rectal lesions (case 2) was biopsied with suspicion of lesions in the context of inflammatory bowel disease. The patient with multiple lesions on the glans penis had an initial clinical diagnosis of erosive lichen planus (case 9, Fig. 1A). In patients with skin tumors, the clinical diagnoses included Kaposi sarcoma (case 5) and primary cutaneous lymphoma (case 8). These 2 patients (cases 5 and 8) were initially diagnosed as diffuse large B-cell lymphoma (DLBCL) but were reclassified after a second opinion. All other patients were diagnosed as EBVMCU from the outset.

Histopathologic Assessment, Immunohistochemistry, and TCR and IgH Gene Rearrangements

The histopathologic features and immunohistochemical findings of our cases are summarized in Tables 3 and 4, respectively. We classified our cases with respect to 2 histopathologic patterns. In the first of these, the skin lesions (cases 5 and 8) and one of the oral tumors (case 6) showed a diffuse pattern with a dense infiltrate of large cells, most of them with immunoblastic morphology, involving the superficial and deep dermis and extending into the subcutaneous fat, with some additional nodular areas, especially around the vessels (Figs. 2A–D). The 2 skin cases exhibited an angiocentric pattern, with large cells infiltrating the wall of medium-sized arteries, and fibrinoid material in the lumina (Fig. 2A). There were some variable-sized cells with apoptotic nuclei and a coarse radial distribution of their chromatin, with features characteristic of "plasmacytoid" apoptotic cells, especially in case 5 (Fig. 2D). Areas with numerous T lymphocytes and necrosis were also observed, with T cells being present mainly around tumoral nodules (Figs. 2I, L). With respect to the second histopathologic pattern, the other oropharyngeal, rectal and genital lesions showed a polymorphic pattern similar to that originally described in EBVMCU, with a polymorphous infiltrate composed of some large cells with Reed-Sternberg or Hodgkin (RSH)-like features and an accompanying infiltrate with medium-sized lymphocytes, neutrophils, eosinophils, plasma cells, and histiocytes in most cases. These cases showed well demarcated margins surrounded by reactive small T lymphocytes. The oral lesions (cases 1, 4, 6, and 7) showed a pseudocarcinomatous hyperplasia in the adjacent epithelium. The angiotropism of the cells was especially prominent in the lesions of genital mucosa (case 9), where large B cells surrounded and infiltrated small vessels (Figs. 2D–H).

In situ hybridization for EBER showed marked positivity in large B cells and pleomorphic RSH-like cells. EBER-positivity was not present in the surrounding lymphocytes, which, in most cases, delineated the lesion (Figs. 1D, 2G, J).

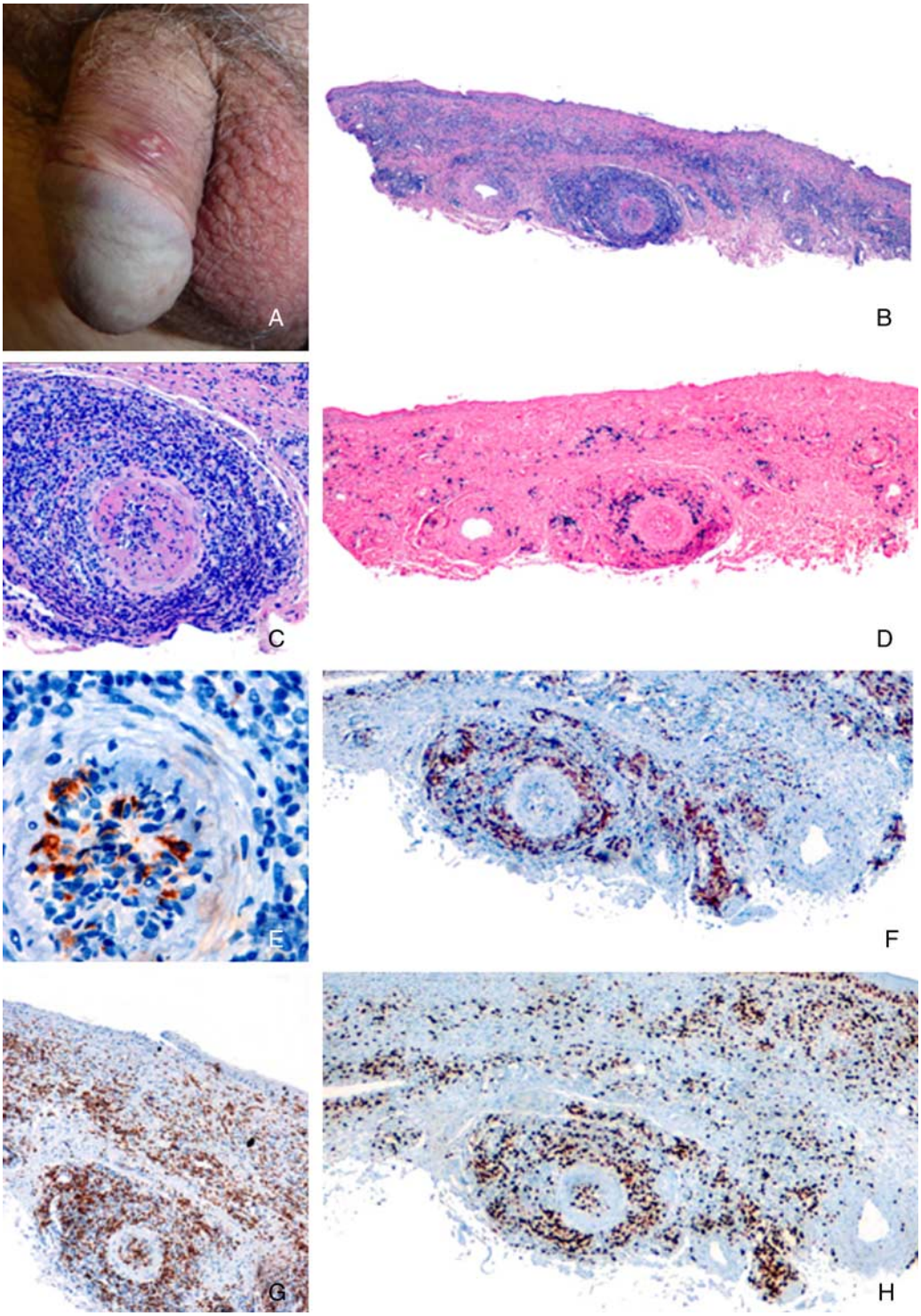

FIGURE 1. Case 9. Multiple genital lesions with a polymorphic and angiotropic histologic pattern. A, Multiple erythematous papules in the glans penis with erosion and a fibrinous background at the base. B, Panoramic view where the infiltrate has an interstitial and perivascular pattern with prominent angioinvasion (H&E). C, Detailed image showing the angiotropism of tumoral cells (H&E). D, EBER-positive large cells scattered in the infiltrate and in the vessel wall (H&E). E, Many of the EBER-positive cells located in the vessel wall expressed CD30. F, Tumoral cells are also positive for CD79a (H&E). G, CD3-positive cells are prominent at the edge of the lesion (H&E). H, Ki67 highlights the tumor cells that have the highest proliferative activity.

TABLE 3. Main Histologic Features of Cases

Case Number	Ulceration/ Necrosis	Reed-Sternberg-like Cells	Perivascular Infiltrate	Angiotropism	Plasma Cells	Histiocytes	PMN	T Cell Infiltrate in Margins	EBV+ Epithelial Cells	Other Biopsies With EBV + Cells	Histologicalpattern
1	P	P	P	A	P	P	P	P	A	Some EBV + cells in the gastric mucosa and bone marrow	Polymorphous
2	P focally	P	P	P	P, polyclonal	P	P	P	A	A	Polymorphous
3	P	P	P	A	P	P	P	P	A	A	Polymorphous
4	P	P	P	A	P	P	A	P	P	A	Polymorphous
5	P	P	P	P	A	A	A	P	A	A	Diffuse
6	P	P	P	A	S	S	S	P	A	A	Diffuse
7	P	P	P	A	P	P	P	P	A	A	Polymorphous
8	P	P	P	P	S	P	P	P	A	A	Diffuse
9	P	P	P	P	P	P	P	A	A	A	Polymorphous

A indicates absent; P, present; PMN, polymorphonuclear cells (neutrophils and/or eosinophils); S, scattered.

Scattered EBER-positive large cells were seen in the vascular walls of the cases with angioinvasion (Fig. 1D). A remarkable finding in case 4 was the presence of EBER-positive epithelial cells in the margins of the EBVMCU lesion (Fig. 3). In addition, while reviewing biopsies performed on the patient for staging, we found EBER-positive cells in the cavum, stomach and bone marrow, without evidence of tumor infiltration in these tissues.

The main immunophenotypic features of EBER-positive large cells are described in Table 3. Large cells and RSH-like cells were strongly positive for CD30 and had an immunophenotype of B cells, with positivity for most of the B-cell markers analyzed here: Pax-5, CD79a, and CD20. Three cases with Pax5 positivity were negative for CD20 (cases 1, 7, and 9). CD20 staining was diffusely positive in the cases with diffuse pattern simulating DLBCL (Figs. 2B, E). Furthermore, CD15 immunostaining was only performed in 5 of the patients, proving to be positive in 2 of them (cases 3 and 4). Bcl-2 was positive in 3 of the 8 cases tested (cases 1, 5, and 6) and MUM1 was positive in all cases in which staining was performed. Some of the smaller cells were also positive for CD30, CD20, Pax5, and CD79a. In the cases tested for PDL1 expression (cases 1, 3, and 4), this marker was found to be positive in large cells and RSH-like cells (Fig. 4), while PD1 was negative. The accompanying cells were mostly positive for CD3 and PD1, delineating the margins of the lesion in some cases (Figs. 1G, 2I).

The proliferative index, measured as counts of the Ki67 marker in EBER-positive cells, was moderate (25% to 50% positive cells) or high (> 50% positive cells) in all cases, whereas the index was low (< 25% positive cells) in EBER-negative cells of the infiltrate (Figs. 1H, 2H, K).

A PCR result with polyclonal TCR gamma and IgH gene rearrangement was obtained in cases 1 and 9. A monoclonal IgH gene rearrangement was obtained in case 8. The other cases were inconclusive or the technique was not performed.

Therapy and Outcome

Follow-up information was available for all patients (Table 2). Of the 7 patients with external visible lesions, one (case 9) had multiple outbreaks with lesions in the genital area that finally resolved spontaneously. In the 2 patients whose lesions were related to their treatment with methotrexate (cases 4 and 6), the lesions resolved when the drug was discontinued. One of the patients died from congestive heart failure (case 5); at the time of death erythematous-violaceous nodules were present on her thigh. The HIV patient with multiple ulcerated CD20-negative lesions, was treated with 4 cycles of CHOP chemotherapy (cyclophosphamide, doxorubicin, vincristine, prednisone) because of the bleeding episodes, and attained complete remission. The second patient to receive chemotherapy (rituximab and bendamustine) (case 8) had a solitary cutaneous lesion, which completely disappeared after treatment was withdrawn. The patient with the single ulcerated tumor located on the floor of their mouth (case 7) was treated with radiotherapy due to their disabling pain, and achieved complete remission. To conclude the clinical description, the

TABLE 4. Main Immunophenotypical Features of Large Cells

Case Number	CD79a	CD20	CD30	Bcl-2	AE1-AE3	PAX5	MUM1	CD15	MIB1-Ki67	CD3	PDL1	PD1	EBER	PAX5/PDL1
1	+	−	+	+	NP	+	+	−	+	−	+	− (+accom)	+	+
2	NP	+	+	NP	NP	NP	NP	NP	NP	−	NP	NP	+	NP
3	+	+	+	−	NP	+	+	+	+	−	+	− (+accom)	+	+
4	+	+	+	−	−	+	+	+	+	−	+	− (+accom)	+	+
5	+	+	+	+	NP	NP	+	NP	+	−	NP	NP	+	NP
6	NP	+	+	+	NP	NP	NP	NP	+	−	NP	NP	+	NP
7	NP	−	+	−	−	+	+	−	+	−	NP	NP	+	NP
8	+	+	+	−	NP	+	+	NP	+	−			+	NP
9	+	−	+F	−	−	+W	+	−	+	−			+	NP

Ki67-MIB1+: > 25% of the cells expressed Ki67.
Accom indicates accompanying reactive T cells; F, focally; NP, not performed; W, weak.

2 patients with rectal lesions had not been treated. In one of them (case 3), the lesion completely disappeared after multiple biopsies, while in the other one (case 2), a lesion continued to show up in the colonoscopy after 6 months, but exhibited no bleeding or symptomatology.

DISCUSSION

This series showed the high variability of clinical presentation and histopathologic features that may be seen in EBVMCU. This series expands the clinical spectrum of EBVMCU, and we describe for the first time its presentation as multiple genital lesions. In previously described cases, the clinical presentation as a solitary lesion was the commonest feature, with ~17% of cases being multifocal.[3] In addition, in our series there were 5 multifocal cases, with similar locations to those previously described, the most frequent being the oropharyngeal region, followed by the skin and intestinal mucosa.[3] Interestingly, all patients responded favorably to the received therapy, thus highlighting the need to recognize the clinical and histopathologic spectrum of the disorder.

From the histopathologic point of view, the recognition in our series of a spectrum of lesions ranging from a polymorphic infiltrate to a more diffuse pattern (large B-cell lymphoma–like), was previously pointed out by Rausch et al,[4] and should be taken into account in future revisions of the classification of EBV-related lymphoproliferative processes. A review of the literature demonstrates that intestinal cases and those located in the oral cavity have a more polymorphic pattern, with the presence of RSH-like cells, an important inflammatory microenvironment composed of histiocytes, polymorphonuclear cells, plasma cells and a CD3-positive T-cell infiltrate arranged in a band, which limits the lesion. This polymorphic pattern is considered highly characteristic of EBVMCU in the oral cavity.[28] The angiocentric pattern, with EBV-positive cells involving the walls of medium-sized vessels, was thought to be a specific characteristic of lymphomatoid granulomatosis.[29–31] However, our study demonstrates that it is also a frequent finding in cases of EBVMCU, especially in those located in the skin and genital mucosa and in the case of the HIV-positive patient with multiple oropharyngeal lesions. The absence of previous low-grade lesions, the absence of clinical pulmonary or CNS involvement proved in CT images, as well as the indolent clinical course of our patients, all provide contribute to the differential diagnosis of these EBV-related entities, particularly with lymphomatoid granulomatosis.[29–31] Other entities whose histologic and clinical features overlap with those of EBVMCU, especially those localized cases arising in the oral cavity, are EBV-associated polymorphic lymphoid proliferations resembling polymorphic posttransplant lymphoproliferative disorders in HIV-positive patients and plasmablastic lymphoma (PBL) located in the oral cavity of HIV patients with DLBCL morphology and CD20-negative tumor cells.[1,32] However, in contrast to EBVMCU, PBL located in the oral cavity of HIV patients usually has an aggressive course with frequent involvement of bone marrow at presentation and dissemination to other locations, with bone involvement in up to 30% of cases, and paraprotein detected in the blood of some patients.[1] From the histopathologic point of view, PBL has a diffuse and monomorphous immunoblastic pattern, unlike EBVMCU of the oral cavity which has a more polymorphous pattern, with abundant accompanying reactive cells.[1] Moreover, PAX5 is negative in PBL and positive in EBVMCU, while cytoplasmic immunoglobulin is found in PBL, unlike in EBVMCU.[1] Besides this, in some cases a differential diagnosis with a Hodgkin lymphoma variant of Richter transformation was raised, as 2 of the patients had a previous history of chronic lymphocytic leukemia.[33]

So far, EBVMCU has been described in different immunosuppression contexts such as immunosuppressive therapy for autoimmune disorders and inflammatory bowel disease under treatment with methotrexate, cyclosporine or azathioprine,[2–26] in organ transplant recipients,[10] after other lymphomas or tumors in the elderly[2–26] and, more recently, in patients with primary immunodeficiencies and in HIV-positive patients.[27] The evolution and the clinicopathologic characteristics are similar in all these aforementioned contexts.

Daroontum and colleagues have recently reported a series comparing the EBVMCU associated with previous lymphoma treatment and those associated with methotrexate. They failed to find any clinicopathologic differences between the 2 entities, although they noted that patients with previous lymphomas had worse prognosis with lower survival due to causes other than MCU.[25] They studied the expression of PDL1 in these lesions for the first time, observing that the macrophages of the microenvironment

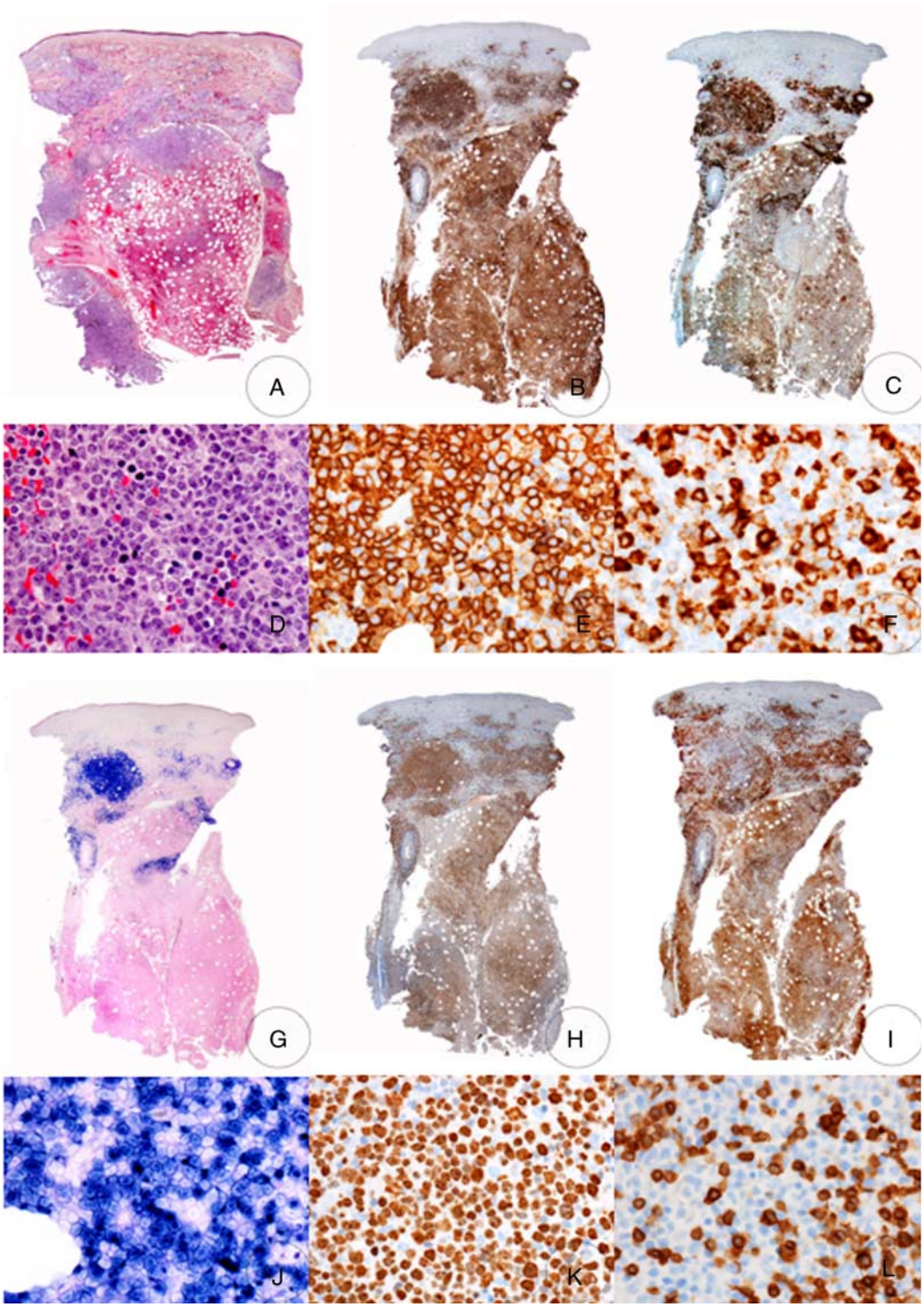

FIGURE 2. Case 5. Tumoral lesions in the skin with a diffuse histologic pattern. A, Low-power magnification of the skin biopsy showing diffuse dermal and subcutaneous infiltration, characterized by a dense infiltrate with prominent large cells (H&E). D, Detail of large, inmunoblastic-like cells present in the infiltrate. Neoplastic cells are positive for CD20 (B, E), CD30 (C, F), and EBER (G, J). Ki67 had a high proliferative index (H, K). CD3 showed a background of T cells (I, L).

were PDL1-positive, while the tumor cells were negative, unlike what occurs in the EBV-positive DLBCL, in which tumoral cells are PDL1-positive.[25] According to these authors, this feature might be related to a lower capacity of these injuries to escape from the immune system.[34,35] However, in our cases we found PDL1-positive tumoral

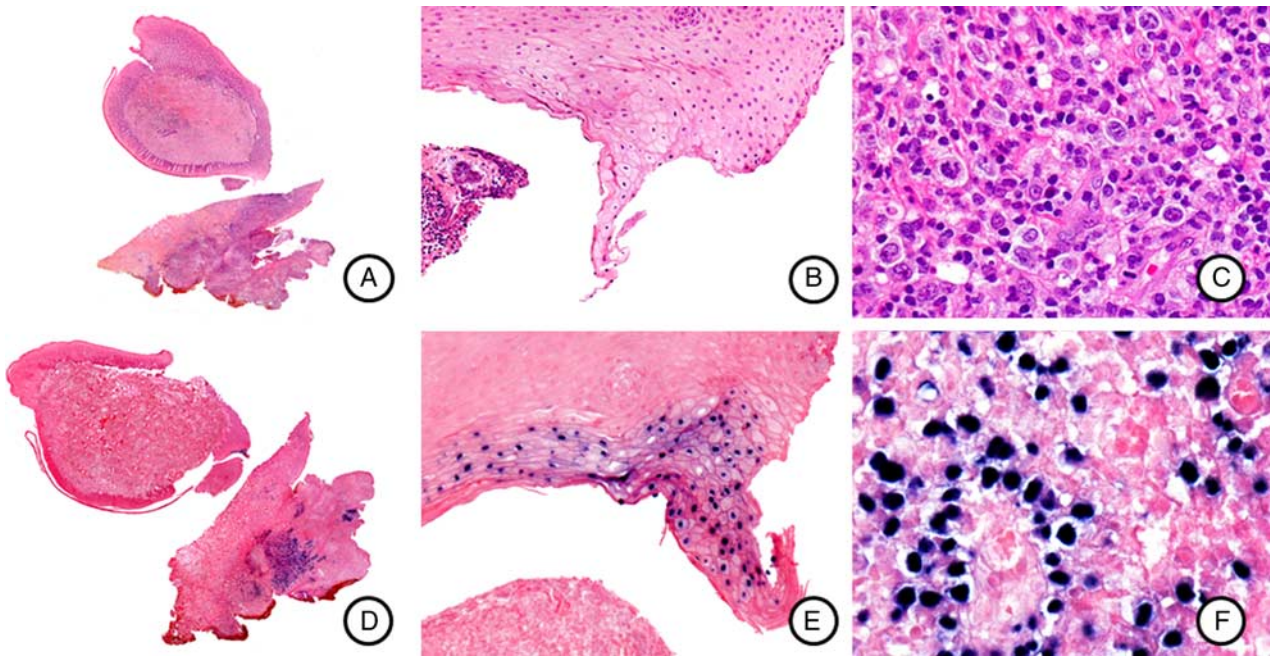

FIGURE 3. Case 4. Oral mucosa located next to EBV-positive MCU showing EBER-positive epithelial cells. Panoramic view with H&E (A) and EBER (D) showing the general pattern of this oral EBVMCU. Detail of epithelial cells with H&E (B) and EBER (E). Large EBER-positive cells in the tumor infiltrate with H&E (C) and EBER (F).

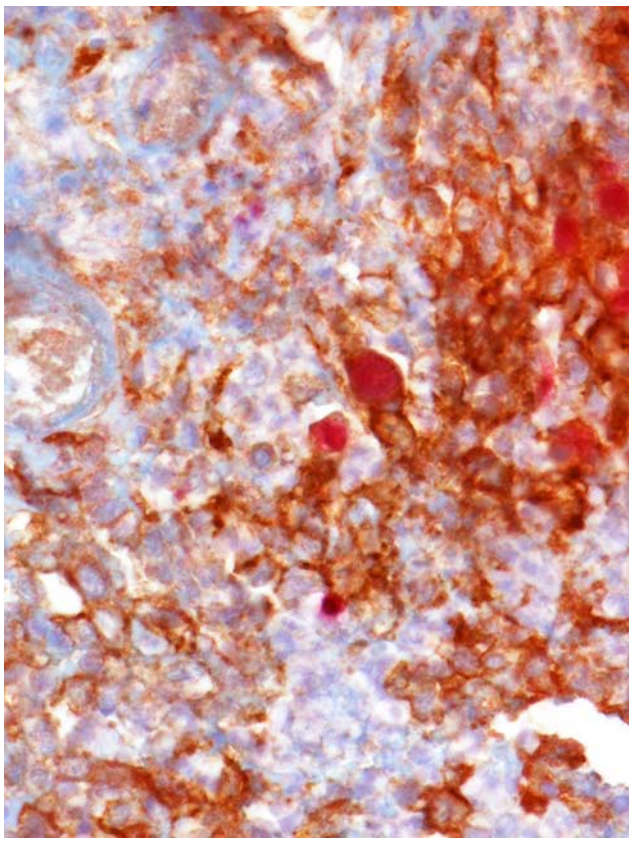

FIGURE 4. Case 1. Double-immunostaining with PDL1 (antibody 28.8) and Pax5, showing some positive tumor cells.

cells that were highlighted with the double PAX5-PDL1 immunostain (Fig. 4). These differences could be due to the different antibodies used for detecting PDL1.

In the first description of EBVMCU the authors did not find any signs of EBV infection in the underlying epithelium.[2] Thus, our case 4 is the first reported instance of infection of the epithelium adjacent to the EBVMCU, as highlighted by its EBER positivity. EBV has been found in epithelial cells of undifferentiated nasopharyngeal carcinoma, oral hairy leukoplakia, some gastric carcinomas, and in the gingival epithelial cells of periodontium in chronic periodontitis.[36,37] It is thought that epithelial infection by EBV in the setting of asymptomatic persistent infection could be a feature of the normal EBV life-cycle and that it could favor the appearance of EBVMCU lesions in these locations where the virus is located.[38] Some authors believe that the absence of systemic activation of the virus and, therefore, the absence of viremia, favors the diagnosis of EBVMCU over other EBV-related lymphoproliferative disorders, supporting the role of the virus that is confined to the mucosa in the pathogenesis of the disease.[10,25] However, we believe that in the absence of systemic disease, if the lesion fulfills the clinical and histopathologic criteria of EBVMCU, the presence of EBV DNA in the blood does not exclude the possibility of that diagnosis. Our case 1 had EBV-positive cells in an earlier biopsy of inflammatory gastritis, normal cavum and bone marrow. DNA of EBV was found at low levels (84 copies/mL), in the blood of this patient. EBV was quantified in whole blood by PCR, using primers that hybridize the EBNA1 protein gene. However, tumor infiltrate was not found in these locations by histopathology or when a

PET-CT scan was performed, fulfilling the diagnostic criteria for EBVMCU.

The molecular study of these lesions may be difficult, especially in cases located on intestinal mucosa, whose infiltrate usually contains few large cells. To differentiate EBVMCU from DLBCL of the oral cavity, Ohata el al[29] performed a mutational panel that included *MYD88, CD79A, CD79B, CARD11*, and *EZH2*, and found that 33.3% of EBV-negative cases harbored at least one mutation, while no gene mutations were observed in the EBV-positive group. They also discussed the complete absence of the germinal center phenotype in EBVMCU, with all lesions being CD10-negative and Bcl6-negative, unlike the cases of EBV-negative DLBCL.[29] IgH and TCR gene rearrangement have been widely studied since the publication of the original series.[2,10,20] There are cases with clonal IgH, cases with clonal and oligoclonal TCR, and cases that are polyclonal for both. Although the neoplasm is considered to be of B-cell origin, the presence of polyclonal or oligoclonal TCR may be related to a restricted T response, as is typical in patients of advanced age.[2] However, until now, the presence of monoclonal or oligoclonal TCR has not been found to be related to a specific type of immunosuppression.[2] In the histopathologic spectrum of EBVMCU lesions, it may be easier to find a monoclonal rearrangement for the IgH gene and an immunophenotype similar to EBV-positive DLBCL in tumoral lesions with more prominent CD20-positive large cell populations.

In conclusion, it is essential to correctly define the clinical and histopathologic spectrum of EBVMCU and identify the factors that condition its evolution and its need for treatment in order to provide better diagnosis and management of these patients, particularly as many of them are of advanced age and for whom aggressive treatments may entail a greater possibility of iatrogenia. Our series includes the genital mucosa between the possible EBVMCU locations and reveals that some lesions previously diagnosed as DLBCL on the basis of histopathological findings could fall within the clinical and histopathologic spectrum of EBVMCU. The role of PDL1 expression in tumor and microenvironmental cells, the importance of epithelial EBV-infected cells, and the presence of EBV DNA in blood samples of these patients should be investigated further.

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Large Cells With CD30 Expression and Hodgkin-like Features in Primary Cutaneous Marginal Zone B-Cell Lymphoma

A Study of 13 Cases

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Abstract: The presence of $CD30^+$ cells in cutaneous lymphomas has come to prominence in recent years as a potential diagnostic and therapeutic marker. In primary cutaneous marginal zone B-cell lymphomas, the presence of large $CD30^+$ cells with Hodgkin-like features and their significance have not yet been studied. Here we describe the main clinical, histologic, immunophenotypic, and molecular characteristics of 13 cases of primary cutaneous marginal zone lymphomas featuring $>10\%$ of $CD30^+$ large cells, and analyze their relationship with histologic and clinical progression of the disease and with other morphologic and immunophenotypic features. We report 10 male and 3 female patients, 4 with early-local disease and 8 with locoregional advanced disease without extracutaneous involvement but with a high relapse rate of 69%. We describe an association between a high level of CD30 expression and disease progression, with increased clinical recurrence in cases with $>15\%$ of $CD30^+$ cells. We also discuss the differential diagnosis with other cutaneous and systemic lymphomas, especially Hodgkin lymphoma.

Key Words: primary cutaneous marginal zone lymphoma, cutaneous-MALT lymphoma, CD30 expression, Hodgkin-like cells, progression

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Primary cutaneous marginal zone lymphoma (PCMZL) is a distinct subtype of indolent cutaneous B-cell lymphoma included in the group of extranodal marginal zone lymphoma of mucosa-associated lymphoid tissue (MALT lymphoma) in the most recent WHO classification.[1] Local recurrence is relatively common in this group of cutaneous B-cell lymphomas, and sporadic cases may show extracutaneous dissemination or large tumoral masses.[2] There are currently very few prognostic markers in PCMZL that are of use for differentiating patients whose disease will have a more aggressive course and who might benefit from systemic treatments.[3,4]

During routine diagnosis of PCMZL, we have frequently observed the presence of $CD30^+$ large cells, sometimes with Hodgkin-like morphology, and, occasionally, even with histologic features mimicking Hodgkin lymphoma.

In this study, our aim was to investigate the presence of $CD30^+$ large cells and Hodgkin-like cells in a routine series of PCMZLs, and their possible link with progression in PCMZL. We investigate their relationship with the presence of atypical T cells and of TCR and IgH gene rearrangements, and review the main clinical, histologic, and molecular characteristics of all the included cases.

MATERIAL AND METHODS

Case Selection

Cases were identified in the electronic database of the Pathology Department of the Fundación Jiménez Diaz University Hospital of Madrid, Spain. We retrospectively reviewed all available skin biopsies, including those performed in our hospital and cases sent for consultation between January 2000 and November 2018. To select the cases, we used a search strategy that included the keywords "CUTANEOUS MARGINAL ZONE LYMPHOMA," "MALT," "CD30," "PROGRESSION," "P53," "LARGE CELLS," "REED-STERNBERG CELLS," and "HODGKIN CELLS." Most of the cases found were of challenging

nature, sent for consultation from various Spanish hospitals during this period. All cases featuring $\geq 10\%$ $CD30^+$ large cells were included. Most of them had cells with morphological features of large pleomorphic cells, including cells with Reed-Sternberg–like or Hodgkin-like morphology. The diagnosis of cutaneous marginal zone lymphoma was always based on the clinical, histologic, and molecular characteristics that define the entity according to the most recent (2017) WHO classification.[1] None of the patients had a previous diagnosis of systemic Hodgkin lymphoma. Additional information collected about patients included age, sex, number of lesions present when the studied biopsy was performed, clinical staging system according to the European Organization for Research and Treatment of Cancer (EORTC)/International Society for Cutaneous Lymphomas (ISCL),[5] the occurrence of cutaneous relapses, previous diagnosis of other lymphomas, treatment performed, and time of follow-up (Table 1).

Histopathologic Assessment, Immunohistochemistry, and In Situ Hybridization

We used formalin-fixed, paraffin-embedded (FFPE) skin biopsies for histopathologic examination. All the skin samples were reviewed, and those of patients' most recent relapse (more progressive disease) were selected to examine the histology and immunostaining. A longitudinal comparative analysis was performed for patients with multiple serial samples. The histologic pattern in all biopsies was classified as one of the following: perivascular/periadnexal, nodular, diffuse, or mixed. In addition, the relative proportion of large $CD30^+$ tumoral cells was visually estimated as the ratio of $CD30^+$ tumor cells to $CD20^+$ tumor cells, and the pattern of CD30 staining was categorized as scattered, clustered, or diffuse. The presence of reactive follicles with germinal centers was assessed morphologically and by Bcl6, Bcl2, and CD21 staining. The presence of light-chain restrictions was evaluated in the plasma cells, and the dominant heavy chain was evaluated in plasma cells and in tumoral B cells; if there were reactive follicles present, these B cells were not included. The following immunohistochemical stainings (obtained from DAKO except where indicated) were performed: CD30, CD15, CD20, CD3, PD1, κ, λ, IgG, IgM, IgD, IgA, CD123, Bcl6, Bcl2, p53, Pax5, CD21, CD23, CD5, Ki67, EBER (Ventana), MYC, and pSTAT3. The proliferation index was assessed as the percentage of tumoral $Ki67^+$; if

TABLE 1. Clinical Data

Case	Age (at Last Relapse) (y)	Sex	No. Lesions (at Last Relapse)	Locations	EORTC/ ISCL Staging	Cutaneous Relapses	Extracutaneous Relapses	Other Lymphomas	Treatment	Follow-up
1	44	M	1	Right arm	T1a	No	No	No	Surgical excision	3 mo
2	51	M	1	Cheek	T2	Yes, 2	No	No	Surgical excision	3 y
3	71	F	3 (masses)	Breast/chest Hip Thigh	T3b	Yes, 3	No	No	R-CHOP(x4)	11 y
4	82	F	3	Both shoulders	T2c	Yes, 2	No	No	Surgical excision	3 y
5	59	F	1	Bottom	T1a	No	No	No	Surgical excision	1 y
6	37	M	1	Back	T1a	No	No	No	Surgical excision	3 y
7	54	M	1	Back	T1a	No	No	No	Surgical excision	1 y
8	35	M	6	Coccyx Right thigh Left leg Right sole (2) Left ankle	T2c	Yes, 3	No	No	Rt, rituximab	5 y
9	75	M	2	Left scapula	T2a	Yes, 2	No	Yes, testicular diffuse large B-cell lymphoma (2011)	Surgical excision	3 mo
10	72	M	>10	Back	T2c	Yes, multiple >4	No	No	Surgical excision Intralesional Rituximab	3 y
11	72	M	3	Left arm	T2c	Yes, multiple >10	No	No	Surgical excision, CE intralesional RTX intralesional RT	24 y
12	68	M	4	Right thigh Back	T2c	Yes, 4	No	No	Surgical excision	3 y
13	39	M	3	Left arm	T2b	Yes, 3	No	No	CE intralesional Topical CE	15 mo

EORTC/ISCL classification (ref): T1, solitary lesion (T1a: <5 cm in diameter, T1b: > 5 cm); T2, regional skin involvement, multiple lesions affecting one body region or two contiguous body regions (T2a: all disease <15 cm diameter circular area: T2b: > 15 and <30 cm; T2c > 30 cm); and T3, generalized skin lesions (T3a: multiple lesions affecting 2 noncontiguous body regions; T3b: multiple lesions involving ≥3 body regions).

F indicates female; M, male.

reactive follicles were present, germinal center proliferative cells were not included.

Polymerase Chain Reaction for IgH and TCR Gene Rearrangement and Other Genotypic Studies

We extracted genomic DNA from tumoral FFPE samples using a RecoverAll Multi-Sample RNA/DNA kit (Invitrogen, Carlsbad, CA) in accordance with the manufacturer's protocol. DNAs and RNAs were quantified with Qubit (Invitrogen).

Polymerase chain reaction (PCR) was performed to analyze the clonal expansion of B and T cells. DNA was extracted from paraffin sections, and T-cell and B-cell clonal expansion was detected by PCR for the immunoglobulin and T-cell receptor gene recombinations.[6] Appropriate positive and negative controls were included in all experiments. Clonality was assayed following well-established recommendations.[7] Each PCR amplification was carried out twice.

To detect the presence of the *MYD88* mutation, we used the primers described by Jiménez et al[8] and quantitative PCR (qPCR) based on allelic discrimination. Reactions were performed in triplicate in a Light Cycler 480 Real-Time PCR System (Roche). Assays were run in 384-well plates in a reaction volume of 10 μL, using 50 ng of genomic DNA (gDNA), 5 μL of Light Cycler 480 Probes Master, 0.15 μL primer (20 μM, wild type or mutated), and 0.1 μL probe (20 μM). The reaction mixture was incubated at 95°C for 10 minutes, followed by 45 cycles of amplification at 95°C for 10 seconds and 60°C for 30 seconds. The data were analyzed with Light Cycler 480 SW 1.5 software (Roche) using the ΔCp method. Cp values were analyzed and showed a SD of <0.25. Cases with a value of Cp >20 were considered wild type.

In cases with enough DNA of suitable quality, a custom panel of genes usually associated with low-grade B-cell lymphomas was performed. The panel included the following target genes: *TP53*, *ARID1A*, *BRAF*, *WHSC1*, *PLCB1*, *SPEN*, *MEF2B*, *MYD88*, *CCND3*, *MAP2K1*, *CCND1*, *MAP3K14*, *NFKBIE*, *MLL2*, *XPO1*, *CHD2*, *BTK*, *BIRC3*, *ATM*, *NOTCH1*, *NOTCH2*, *POT1*, *CXCR4*, *TNFAIP3*, *SF3B1*, *KLF2*, and *TLR2*. Dual-strand sequencing eliminated false-positive C-T mutations that can arise from deamination events during formalin fixation. The probes for this custom panel were designed using DesignStudio (Illumina) and consisted of 1287 amplicons with an average size of 175 bp and a cumulative targeted region of 140 kb. Polymorphisms were avoided in the design of the primers. When only one strand could be evaluated, we only validated the mutation if it was present at the same extent in another lesion sample from the same patient.

Target enrichment was performed in FFPE-extracted DNA according to the manufacturer's instructions (TruSeq Amplicon—Cancer Panel Library Preparation Guide; January 2017; Illumina). The total amount of input DNA ranged from 30 to 100 ng. After library preparation, indexing, and bead purification, the libraries (2 per sample, 1 per strand) were quantified by Qubit (Thermo Fisher Scientific), normalized with beads, and pooled for sequencing. The pooled libraries were sequenced with a Miseq Reagent Kit V2 (paired-end, 2×151) on a MiSeq instrument (Illumina), as directed in the manufacturer's protocol.

Statistical Evaluation

Statistical analyses consisted of Fisher exact tests (2-tailed *P*-values) and 1-way analysis of variance, as appropriate, carried out in STATA version 14.2.

RESULTS

Clinical Presentation

Clinical information was available for all 13 patients in our series. Age at presentation ranged from 30 to 79 years, with a median age of 54 years. Male individuals predominated in the sample (10 male patients, 3 female patients). The lesions were clinically described as nodules, agminated papules, raised plaques, or, in one case (case 3), large tumoral masses (Fig. 1). Following the staging system of the European Organization for Research and Treatment of Cancer (EORTC)/International Society for Cutaneous Lymphomas (ISCL),[5] 4 patients in our series (cases 1, 5, 6, and 7) had early located disease, and the others had more advanced disease: 8 locoregional cases (patients 2, 4, 8, 9, 10, 11, 12, and 13) and 1 generalized case (patient 3). The lesions were most often located in the extremities (5 patients), followed by trunk (4 patients), trunk and extremities (3 patients), and face (1 patient). None of the patients showed extracutaneous involvement in image tests (TAC in most patients, and PET-TAC in cases 3 and 9) or bone marrow biopsies performed in all cases except patients 1, 5, 6, and 7 in this series. None of the patients had a history of systemic Hodgkin lymphoma. Especially in case 11, who exhibited Hodgkin-like cells positive for CD30 and CD15, a new extension study was performed when the morphology of the PCMZL changed to a more diffuse, Hodgkin-like pattern with no evident extracutaneous disease. Patient 9 had a previous history of testicular diffuse large B-cell lymphoma with an activated phenotype diagnosed 7 years before and treated with R-CHOP, and he achieved a sustained complete response. When, some months before, the cutaneous nodule diagnosed with PCMZL had been excised, a new, complete extension study was made, which showed no evidence of extracutaneous disease. The follow-up time for patients ranged from 3 months to 24 years, the average being 54 months. To date, no deaths have been reported in the series.

Histopathologic and Immunophenotypic Features

Eleven cases in our series showed a predominant nodular pattern, and only 2 (cases 3 and 11) had a clearly diffuse distribution, both of which showed a nodular pattern in the previous diagnostic samples (Fig. 2).

None of our cases showed epitheliotropism or lymphoepithelial lesions. All cases had a diffuse T infiltrate, most of which (69%; cases 4, 5, 6, 7, 8, 10, 11, 12, and 13) formed T-cell rosettes, which were $PD1^+$ around the $CD30^+$ large cells. The $PD1^+$ T cells had atypical morphology. In

FIGURE 1. Large tumor lesions present in the most recent recurrence of case 3. Right breast (A); left thigh (B); and the scar of a previous lesion treated in the left arm (C).

some of these cases, their size was variable, and they formed small clusters (Fig. 3). Reactive follicles with partially colonized germinal centers were not uncommon, being seen in all our patients except cases 3 and 11. Tumoral cells in our cases were mostly $CD20^+$ B cells, with monotypic plasma cells present, especially at the periphery of the lesions in all but 2 cases. The expression of IgM by tumoral plasma cells does not exclude the presence of reactive follicles (cases 2, 3, 6, 7, and 11) in our series. However, in these cases, the germinal centers were not as evident as in those with class-switched plasma cells (cases 1, 4, 8, 9, 10, 12, and 13).

Tumoral B cells were positive for CD20, Pax5, and Bcl2, and negative for Bcl-6 and CD10 in all cases (Table 3). All cases were $EBER^-$, thus ruling out $CD30^+$ lymphomas related to EBV infection (Table 3). In all cases with sufficient material available for study, tumoral cells were $CD5^-$ and $CD23^-$.

Clusters of $CD123^+$ cells were found in most cases (Table 3), distributed mainly in small perivascular aggregates or clusters, or, less frequently, were scattered. They never comprised > 5% of the total cell infiltrate.

$CD30^+$ cells were scattered, clustered, or occurred diffusely throughout the tumoral infiltrate (Table 2). These cells were $CD15^-$ in all but case 11 (Table 3), and most had immunoblast cytology, occasionally with Hodgkin-like morphology (Table 2; Figs. 2, 3). The 2 cases of histologic progression, which showed an increase in the number of large cells in consecutive samples, underwent a change in the number and pattern of distribution of $CD30^+$ cells (Fig. 2). The first biopsy of case 3 exhibited scattered and small aggregates of $CD30^+$ cells in reactive follicles, and an intralymphatic distribution (Fig. 2J), highlighted by D2-40 staining. The second biopsy, performed during an intermediate relapse of the tumor, showed that these $CD30^+$ cells were located in clusters (Fig. 2K). However, in the last biopsy, considered to be that of histological progression, $CD30^+$ cells were more abundant (40% of tumor cells) and diffusely distributed around the tumor (Fig. 2L). The same diffuse CD30

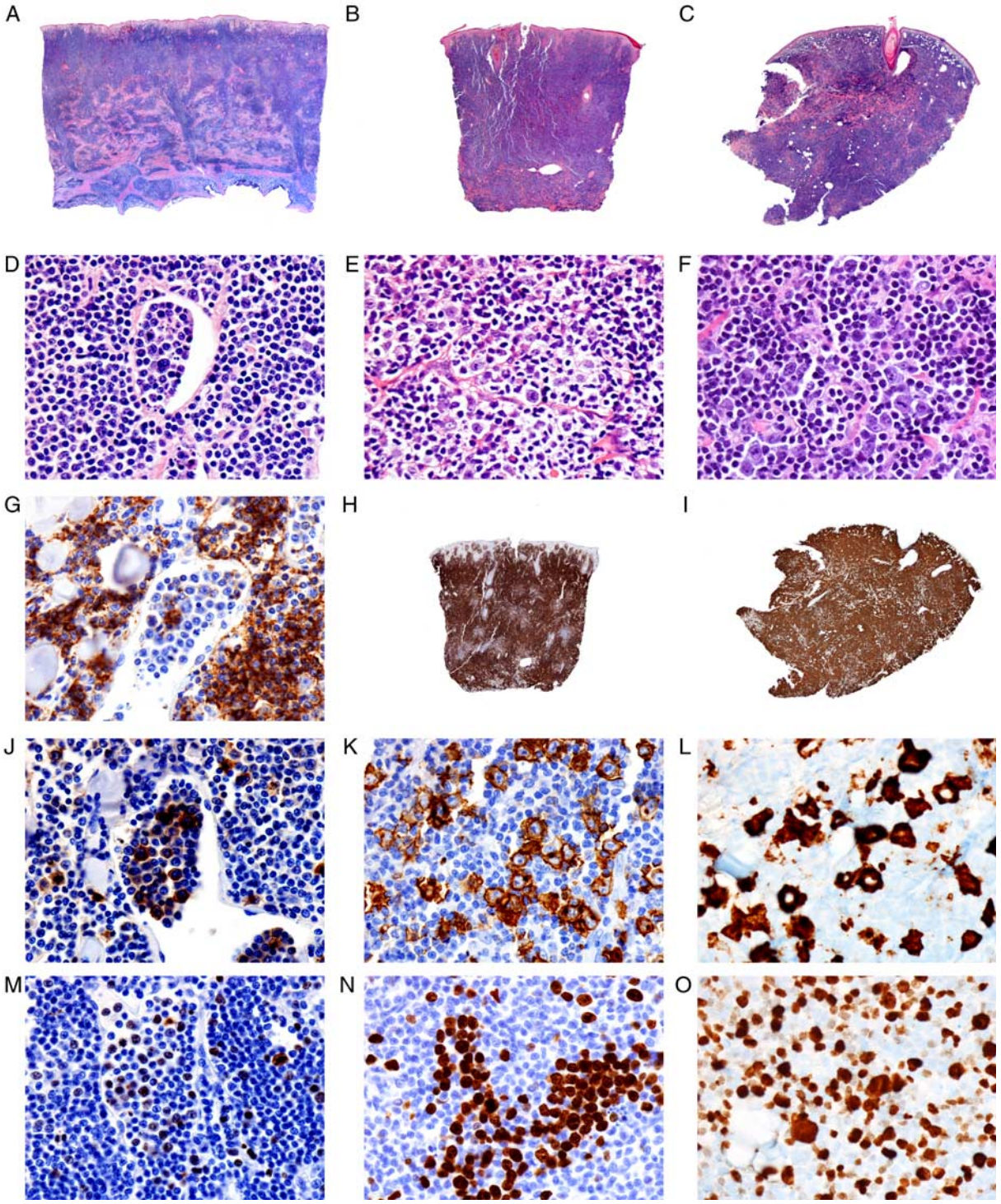

FIGURE 2A. Three samples from patient 3 illustrating the histologic progression of the disease. A, First biopsy (HE) showing (D). Large immunoblasts or Hodgkin-like cells located in a lymphatic vessel (HE) with (G), CD20 (CD20); (J), CD30$^+$ cells (CD30); (M), a low-intermediate proliferation rate (ki67); and (P), a clonal FR3 peak. B, Biopsy of the second relapse (HE) with (E) cytologic detail (HE) and (H) diffuse CD20$^+$ cells (HE) and (K) clusters of CD30$^+$ large cells (CD30) and (N) intermediate Ki67 proliferation rate (Ki67) with (Q) the same FR3 peak. C, Last relapse (HE) with (F) large atypical cells (HE), (I) positive for CD20 (HE) and (L) with 40% of CD30$^+$ large cells located diffusely and interstitially with (O) a high (50%) proliferation rate. It has a common FR3 clonal peak with the previous 2 relapses (R).

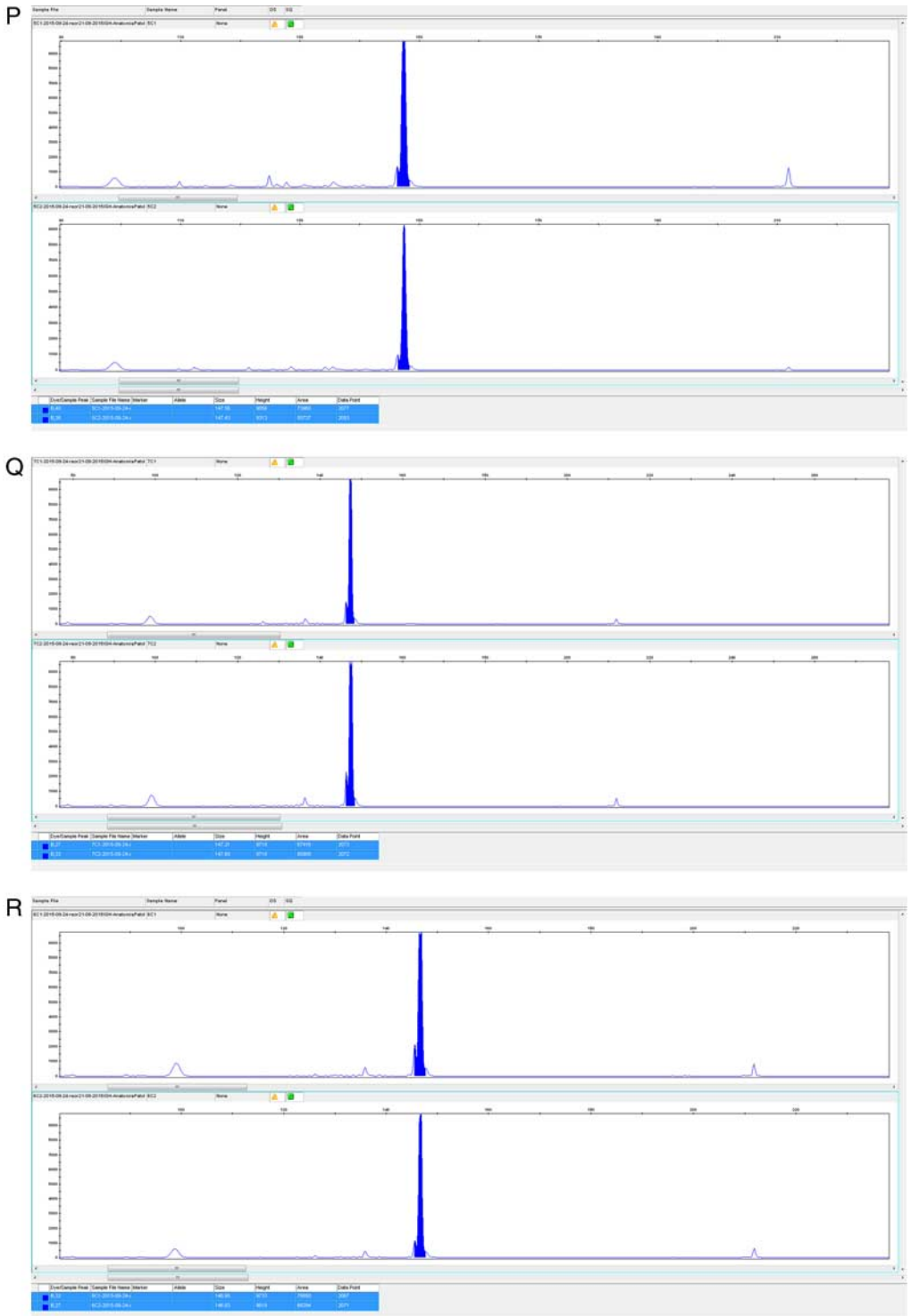

FIGURE 2B. (*continued*).

pattern was observed in the final biopsy of case 11, unlike in the first lesions removed from the patient, in which no CD30 cells, or only a few isolated $CD30^+$ cells, were found in reactive follicles. There was no significant difference in the percentage of $CD30^+$ cells depending on the presence or absence of relapses (analysis of variance, $P > 0.075$). However, comparing the patients with 10% of $CD30^+$ cells with those with at least 15%, showed the latter group to have significantly more relapses ($P = 0.021$). In our series, the expression of IgM or

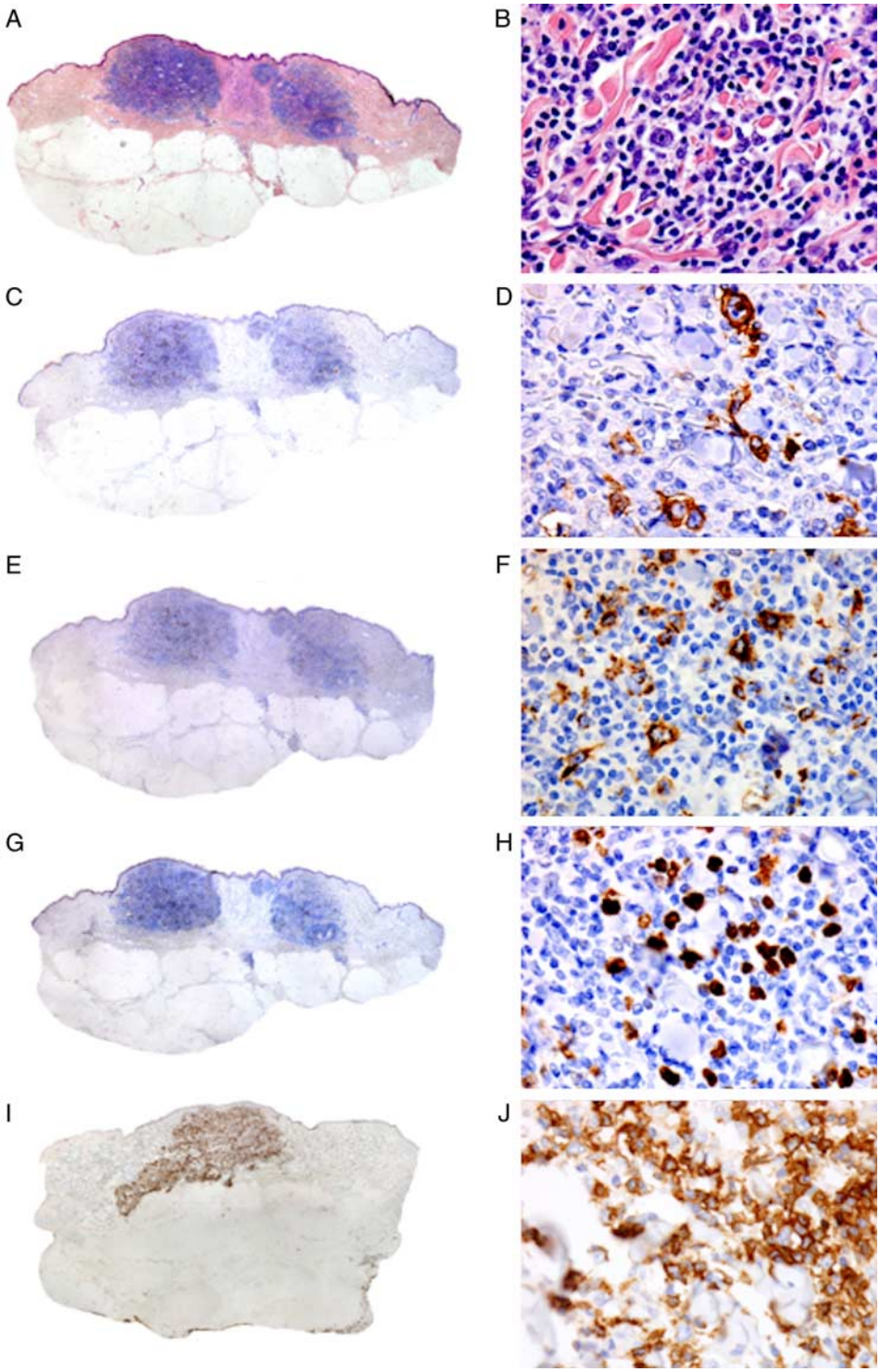

FIGURE 3. Case 11. A, Panoramic view of the tumor infiltrate (HE) with (B) atypical large cells with prominent nucleolus (HE) C and D, CD30 membrane staining in large cells, with CD15 positivity (E) (CD15), (F) (CD15). There are scattered cells with P53 positivity (G) (p53), (H) (p53), and atypical CD3. $PD1^+$ T cells form rosettes or pseudorosettes around $CD30^+$ large cells. I and J, PD1.

class-switched B cells was not associated with the occurrence of relapses ($P = 0.491$) or the presence of $CD30^+$ cells ($P = 0.242$).

In case 11, the $CD30^+$ and $CD15^+$ large cells were also strongly positive for Pax5 and CD20 (Fig. 4), unlike what occurs in Hodgkin lymphoma. pSTAT3 was negative in all

TABLE 2. Main Histologic Features of the Cases

Case	Growth Pattern	Tumor Infiltrate	Reactive Follicles	Inflammatory Background	Presence of Large Cells (%)	$CD30^+$ Cells: Distribution and %	Reed-Sternberg or Hodgkin-like Cells	Epithelio-tropism
1	N	Mostly B and plasma cells	P	T	10-15	10, I	P	A
2	N	Mostly B and plasma cells	P	T	10-15	10, I	P	A
3	D	Mostly B	A*	T	40-50	40, D	P	A
4	N	Mostly B	P	T Rosettes around CD30 cells	30	30, I, D	P	A
5	N	Mostly B and plasma cells	P	T Rosettes around CD30 cells	20-30	10, I	P	A
6	N	Mostly B and plasma cells	P	T Rosettes around CD30 cells	20	10, I	P	A
7	N	Mostly B and plasma cells	P	T Rosettes around CD30 cells	10	10, I	P	A
8	N	Mostly B and plasma cells	P	T Rosettes around CD30 cells	20	15, I	P	A
9	N	Mostly B and plasma cells	P	T	20	15, I	P	A
10	N, M	Mostly B and plasma cells	A*	T Rosettes around CD30 cells	10	10, I	P	A
11	D	B cells	A*	T Rosettes around CD30 cells	30	15, I, D	P	A
12	N	Mostly B and plasma cells	P	T Rosettes around CD30 cells	20	15, I	P	A
13	N	Mostly B and plasma cells	P	T Rosettes around CD30 cells	30	30, I, D	P	A

A* indicates absent but with disrupted follicular structures; D, diffuse; I, interstitial; M, mixed; N, nodular; P, present.

cases in tumor cells, and there were a few scattered large cells that were positive for c-myc. In the 2 cases considered to exhibit disease progression (cases 3 and 11), we found ~20% to 30% of $p53^+$ cells. Ki67 was low or intermediate, ranging from 20% to 50%, in tumor cells of all cases. Samples with a higher proportion of Ki67 corresponded to the progressed samples, not to the original ones.

TCR and IgH Gene Rearrangements and Other Genotypic Studies

All cases in which the technique could be performed had a clonal rearrangement of IgH and/or of light chains in all or most of the available samples (Table 4). The same clonal peak was found in all cases but one (case 12), in which the rearrangement could be carried out in all the samples. Case 12 is exceptional, as the recurrences of the tumor involved a change in light chain restriction. The first lesion removed featured a kappa restriction, and the relapses involved lambda restrictions. The first biopsy with the kappa restriction had a peak of IgH FR2 at 265. However, the biopsy of the lambda recurrence samples showed an IgH FR2 peak at 261, making a 4-bp difference.

In our series, 7 cases had a TCR gamma, beta clonal rearrangement, or both, in addition to B-cell clones (Table 4). All analyzed cases with a more atypical $PD1^+$ T-cell infiltrate displayed a T-cell monoclonal rearrangement.

An L265P *MYD88* mutation was found in case 9.

A customized panel for low-grade B-cell lymphoma NGS was used in all 13 cases. Six cases (cases 2, 5, 7, 10, 12, and 13) were not valuable because there was not enough quality DNA available to perform the technique (Table 4). Case 3 had the same mutations in *KMT2D*-R5048L and *KMT2D*-C349W (*MLL2*) in the initial diagnostic and subsequent relapse samples (Table 4). Cases 1 and 11 had the same *NOTCH2-A3F* mutation, and case 9 had an *NFKBIE* mutation in addition to the *MYD88* L265P mutation. Cases 4, 6, and 8 were wild type.

DISCUSSION

The presence of scattered large neoplastic cells has been noted in the description of PCMZL series,[9–11] and some of these studies have already mentioned the expression of CD30 by these large cells.[12] Since then, there have been few further studies of this finding and its implications for PCMZL. Rodríguez-Pinilla et al[13] described the presence of $CD30^+$ large lymphoid cells surrounded by $PD1^+$ cells in PCMZL. In our series, in addition to what occurs with $CD30^+$ cells in the reactive follicles of PCMZL (Fig. 2H), we

TABLE 3. Main Immunophenotypic Features of Tumoral Cells and Companion Infiltrates

Case	CD20	CD30	CD15	Pax5	EBER	p53	Ki67 (%)	Bcl6	MYC	Bcl2	pSTAT3	CD123 CELLS	Light Chain Restriction	Dominant Plasma Cell Heavy Chain
1	+	+ in large cells	−	+	−	−	20	−	+ in some scattered large cells	+	−	Present clusters	Lamda	IgG
2	+	+	−	+	−	−	20	−	+ in some scattered large cells	+	Scattered	Scattered	Kappa	IgM
3	+	+	−	+	−	+ Scattered	50	−	NP	+	NP	NP	Kappa	IgM
4	+	+	−	+	−	−	20-30	−	+ in some scattered large cells	+	−	Present, clusters	Kappa	IgG-IgG4
5	+	+	−	+	−	−	20-30	−	+ in some scattered large cells	+	−	Present, clusters	No restriction	No plasma cells
6	+	+	−	+	−	−	20	−	+ in some scattered large cells	+	−	Clusters around vessels	Lambda	IgM
7	+	+	−	+	−	−	20	−	+ in some scattered large cells	+	−	Clusters around vessels	Kappa	IgM (Myd88 not mutated)
8	+	+	−	+	−	−	30	−	+ in some scattered large cells	+	−	Clusters around vessels	Kappa	IgG
9	+	+	−	+	−	−	20	−	+ in some scattered large cells	+	−	Clusters around vessels	Kappa	IgG4 Myd88 mutated
10	+	+	−	+	−	−	30	−	+ in some scattered large cells	+	−	Clusters around vessels	Lambda	IgG
11	+	+	+	+	−	+ Scattered	30	−	+ in some scattered large cells	+	−	Clusters around vessels	None	No plasma cells present
12	+	+	−	+	−	−	30	−	+ in some scattered large cells	+	−	Clusters around vessels	Lambda	IgG
13	+	+	−	+	−	−	30	−	+ in large cells, in follicles	+	−	Absent	Kappa	IgG

NP indicates not performed.

found a more diffuse distribution of $CD30^+$ large cells, located outside the reactive follicles and surrounded by $PD1^+$ T cells. As exemplified by cases 3 and 11 of our series, an increase in the frequency of $CD30^+$ large cells could be associated with histological transformation and clinical progression, with a higher frequency of relapses seen in these cases with a higher frequency of $CD30^+$ cells.

The presence of $PD1^+$ T-cell rosettes, noted in most of our cases, could be related to the presence of the TCR-gamma or TCR-beta clonality detected by the TCR studies performed in this series. This has been previously described in PCMZL[14] and in other B-cell lymphomas.[15] Clonal expansion of T cells could well explain this finding.

Several authors have previously described the presence of PD1-expressing T cells. In a group of 6 PCMZLs, Goyal et al[16] found 17% to 34% reactive $CD3^+$ T cells expressing PD1, calculated as the PD1:CD3 ratio, which were lower values than those found in reactive processes. Some years later, Edinger et al[17] again studied the proportion of $PD1^+$ cells in PCMZL and the differences between this disease and primary cutaneous $CD4^+$ small/medium T-cell lymphoproliferative disorder. They found lower proportions (< 10%) of $PD1^+$ cells in PCMZL in all their cases compared with $CD4^+$ small/medium T-cell lymphoproliferative disorder (20% to 30%).[17]

In addition to those previously mentioned, the presence of $CD30^+$ large cells with immunoblast or Hodgkin-like morphology surrounded by rosettes of atypical T cells in our cases led us to rule out the presence of a systemic Hodgkin lymphoma.[18,19] However, such an entity does not exist. The rare cutaneous presentations of cutaneous Hodgkin lymphoma are always secondary to systemic disease, as most cases reported as primary cutaneous Hodgkin lymphoma are actually examples of other cutaneous lymphomas with Hodgkin-like features, such as lymphomatoid papulosis or primary cutaneous anaplastic large cell lymphoma, or, as in our cases, PCMZL with Hodgkin-like cells.[20]

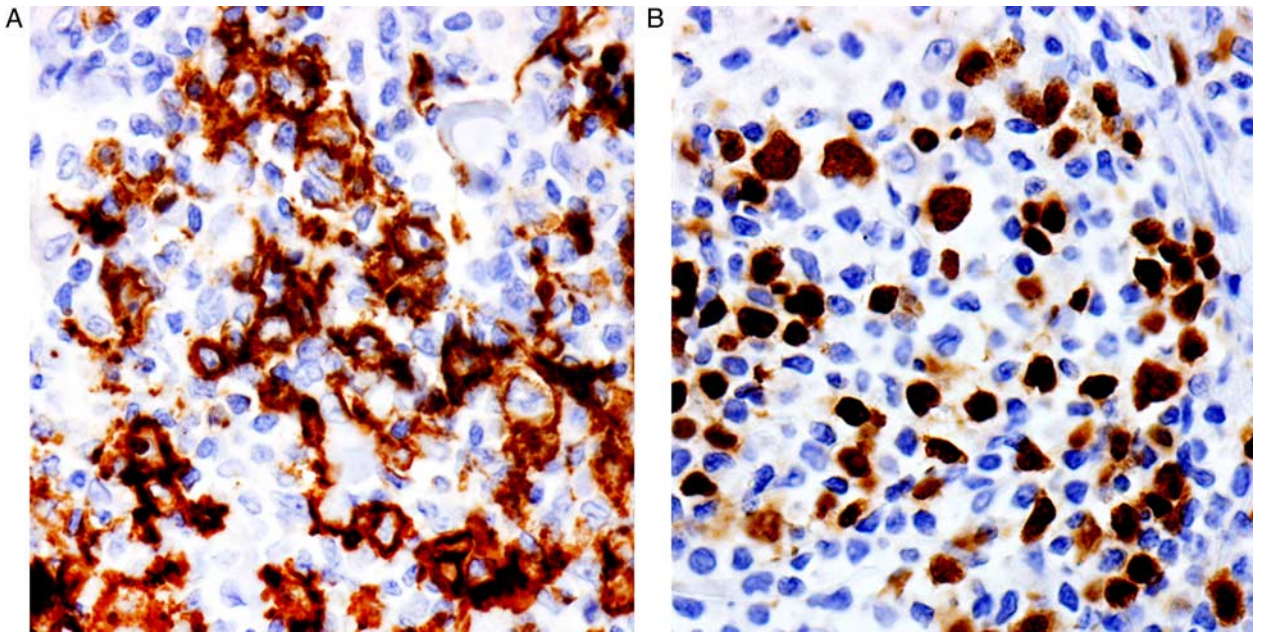

FIGURE 4. Large Hodgkin-like cells with (A) CD20 and (B) PAX5 positivity in case 11 (HE).

The light-chain switch observed in case 12 is a very unusual event in PCMZL. It has only rarely been described in other lymphoma types, like follicular lymphoma[21,22] or posttransplantation lymphoproliferative disease[23] in relation to disease progression and transformation.

TABLE 4. Genotypic Studies

Case	TCR Gamma Rearrangement	TCR Beta Rearrangement	IgH Gene Rearrangement	Kappa, Kappa Del, Lambda Rearrangement	MYD88 L265P Mutation	MALT1 Translocation	Other Mutations Found in Low-grade B-cell Lymphoma Customized Panel
1	Clonal	Clonal	FR1, FR2, FR3 Polyclonal	Ig kappa and kappa del clonal Ig lambda polyclonal	NP	NP	*NOTCH2-A3F*
2	No Clonal	Clonal	FR3 clonal	Ig kappa clonal	NP	NP	NE
3	No clonal	No clonal	FR1, FR2, FR3 clonal	NP	A	NE	*KMT2D-R5048L KMT2D-C349W*
4	Clonal	Clonal	FR1 clonal	Ig kappa clonal Ig lambda and kappa del irregular polyclonal	A	A	WT
5	No clonal	No clonal	FR1, FR3 clonal FR2 pseudoclonal	Ig lambda clonal	NP	NP	NE
6	NP	NP	NP	NP	NP	NP	WT
7	NE	NE	No clonal	Ig kappa clonal	A	NP	NE
8	Clonal	Clonal	Pseudoclonal FR1 Clonal FR2 Polyclonal FR3	Kappa oligoclonal Lambda pseudoclonal Kappa del clonal	NP	NP	WT
9	Clonal	Clonal	FR1, FR2, FR3 clonal	NP	P	NP	*NFKBIE*
10	NE	NE	FR1 clonal FR2, FR3 polyclonal	NP	NP	NP	NE
11	No clonal	Clonal	FR2 clonal FR3 polyclonal	NP	NP	NP	*NOTCH2-A3F*
12	No clonal	Clonal	FR1 and FR2 clonal FR3 polyclonal	NP	NP	NP	NE
13	NE	NE	No clonal	Ig kappa clonal	NP	NP	NE

NE indicates not evaluable, insufficient DNA; NP, not performed; WT, wild type.

The cases described here bear some similarities with those previously denominated as blastic marginal zone lymphoma[24] or diffuse large B-cell lymphoma arising in PCMZL,[11] although here we have included cases showing PCMZL morphology with only a partial increase in the number of large cells, but not those of large cell lymphomas. In addition, CD30 expression was not analyzed in these studies.

Few series with sufficient patients with PCMZL have been studied and followed-up for long enough to estimate the relapse rate and disease-free survival accurately.[3,4,11] One of the largest series is that of Servitje et al,[3] consisting of 137 patients diagnosed with PCMZL, in whom multifocal lesions or T3 disease were the characteristics significantly related to a higher relapse rate and shorter DFS. These patients had similar clinical features to ours, with a predominance of male patients bearing lesions on the trunk and extremities.[3] Like us, they used the EORTC/ISCL staging system,[5] but, unlike our series, most of their patients (51%) were in T1 (T1a, T1b) stage, and 44% of them had suffered relapse.[3] In our series, selected on the basis of the high proportion of large $CD30^+$ cells, the percentage of relapses was higher (69%), and only 31% of patients were in T1 stage. Our series showed a statistically significant relationship between the presence of high frequencies of $CD30^+$ cells and the clinical progression of the disease (more advanced clinical stages and multiple relapses).

Molecular studies in this series revealed mutations in *KMT2D*, *NOTCH2*, *NFKBIE*, and *MYD88* genes. These mutations have been found in other studies of marginal zone lymphoma, singularly in the ocular adnexa marginal zone lymphoma[25] and splenic marginal zone lymphoma,[26] with some IgM-positive cases of PCMZL carrying *MYD88* mutations.[27]

In conclusion, the presence of neoplastic large $CD30^+$ cells is not unusual in PCMZL, wherein they are frequently associated with $PD1^+$ T-cell rosettes. When present, cases with a large number of $CD30^+$ cells exhibited more aggressive behavior, with multiple recurrences in different locations and large tumor masses. For this reason, we think that CD30 should be added to the immunohistochemical panel of those cases of PCMZL with Hodgkin-like cells. It appears to be a good marker of a greater tendency for lesions to recur. To make an accurate differential diagnosis with Hodgkin lymphoma, it must be borne in mind that $CD30^+$ cells are relatively common in PCMZLs, making a full clinical and histopathologic study necessary.

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TERCERA PARTE

III. MEMORIA DE TRABAJO

1. HIPÓTESIS Y OBJETIVOS

HIPÓTESIS

Una clasificación de los linfomas cutáneos con expresión de CD30 que integre datos clínicos, histológicos, inmunofenotípicos y moleculares permite precisar el diagnóstico y predecir mejor el pronóstico de los pacientes, así como seleccionar mejor los candidatos a terapias dirigidas.

OBJETIVOS

1. Revisar las principales características genéticas, epigenéticas y moleculares descritas hasta la fecha en procesos linfoproliferativos CD30 positivos primarios cutáneos y su potencial como dianas terapéuticas.
2. Identificar la histología e inmunofenotipo que caracterizan los linfomas anaplásicos cutáneos con reordenamiento de DUSP22.
3. Estudiar las características clínicas, inmunofenotípicas y el pronóstico de las diferentes lesiones que pueden incluirse en el diagnóstico de UMC-EBV+ por compartir un comportamiento clínico similar, requiriendo tratamientos menos agresivos, recalcando los datos en los que debemos apoyarnos para hacer el diagnostico diferencial con linfomas más agresivos.
4. Estudiar por primera vez las posibles implicaciones clínicas, histológicas, moleculares y en el pronóstico de la expresión de CD30 en los linfomas primarios cutáneos de la zona marginal.

2. APORTACIONES DEL DOCTORANDO

Durante la realización de esta tesis he trabajado activamente en la elección del tema de la tesis, así como de la temática concreta y los casos que se incluyen en cada uno de los artículos. Además, durante la estancia de un año en la Fundación Jiménez Díaz como contrato Rio Ortega bajo la tutela del Dr Miguel Ángel Piris y la Dra Socorro María Rodríguez Pinilla, he participado en la realización de las técnicas moleculares en el laboratorio que se han realizado en los casos de los artículos que componen la tesis, junto a la Bióloga molecular Rebeca Manso y a los técnicos del laboratorio de Biología molecular del Departamento de Anatomía Patológica de la FJD.

Las ilustraciones publicadas en la tabla 2 de este trabajo y en el artículo 1, figura 4, así como en la portada de esta tesis, han sido diseñadas por la doctoranda y elaboradas en formato digital por A. Alzelai diseñadora gráfica que lo ha realizado de forma totalmente desinteresada a partir de dibujos realizados a mano con rotuladores.

A lo largo de esta tesis, he escrito tres de los artículos como primera autora y autora para correspondencia, que incluyen el artículo 1,3 y 4 y he participado en la elaboración y las técnicas descritas anteriormente del artículo 2, cuya autora principal es la Doctora Arantza Onaindia que con gran generosidad ha aceptado aportar parte de su trabajo para completar la elaboración de esta tesis. El análisis estadístico de los datos del artículo 4 ha sido realizado íntegramente por la doctoranda con ayuda de la formación específica impartida en los módulos de estadística aplicada y metodología de la investigación pertenecientes a la Diplomatura en estadística en Ciencias de la Salud impartida en la Universidad Autónoma de Barcelona.

Por último, una vez finalizados y aceptados los 4 artículos para su publicación, he elaborado esta introducción y memoria de trabajo que componen la tesis.

3. METODOLOGÍA

En este apartado se enumeran de forma general los pacientes, recursos, materiales y métodos empleados en el desarrollo de esta tesis doctoral. Este apartado está desarrollado al detalle en la parte correspondiente de los 4 artículos que componen esta tesis doctoral.

REVISIÓN BIBLIOGRÁFICA ARTÍCULO 1

El primer artículo corresponde a una revisión narrativa sobre las alteraciones moleculares y las dianas terapéuticas en los procesos linfoproliferativos CD30+ primarios cutáneos. Para su realización, se llevó a cabo una revisión de la literatura, utilizando la base de datos de MEDLINE, PubMed, utilizando las palabras clave "CD30-POSITIVE PRIMARY CUTANEOUS LYMPHOPROLIFERATIVE DISORDERS", "PRIMARY CUTANEOUS ANAPLASTIC LARGE CELL LYMPHOMA" "LYMPHOMATOID PAPULOSIS". Estos términos arrojaron un total de 150, 622 y 904 resultados respectivamente. Estas palabras clave se cruzaron además con los términos "MOLECULAR ALTERATIONS" y "TARGETED THERAPIES". Además se revisaron estas entidades según la última edición de la clasificación de los linfomas de la OMS ("WHO classificatin of tumors of Haematopoietic and Lymphoid tissues") para seguir en todo momento su terminología y criterios de clasificación. Mediante el título, el resumen y la relevancia y fiabilidad de la revista en la que estaban publicados se seleccionaron finalmente 110 artículos que se leyeron para la realización final del artículo.

MUESTRAS TISULARES

Todas las muestras tisulares de pacientes han sido colectadas del archivo de Anatomía Patológica de la Fundación Jimenez Díaz, así como del biobanco de tejidos del Hospital Universitario Marqués de Valdecilla (HUMV) en colaboración con otros hospitales y con el Centro Nacional de investigaciones Oncológicas (CNIO, Madrid). En el segundo artículo se estudiaron 91 casos con diagnóstico de ALCL, sistémico o primario cutáneo, procedentes del biobanco del HUMV y de la Fundación Jiménez Díaz. De los 91 casos evaluados, 18 eran pcALCL y 71 eran ALCL sistémicos, de los cuales 19 eran ALCL ALK+ y se desestimaron para seguir el estudio. Se han utilizado muestras tisulares en fresco y parafinadas tanto ganglionares como de partes blandas y cutáneas. Sólo 31 casos pudieron seleccionarse para la realización de técnicas FISH por tener suficiente material de suficiente calidad para poder realizar la técnica, estos incluían 22 ALCLs ALK- , 9 pcALCL. En el tercer artículo se estudiaron 9 casos, en muestras parafinadas de piel, mucosa oral y genital, así como mucosa gastrointestinal, de UMC-EBV+ procedentes de la Fundación Jiménez Díaz, que incluían casos remitidos en consulta desde el Hospital Universitario de Guadalajara, el Hospital Rey Juan Carlos de Móstoles y el Hospital General de Villalba. Por último en el cuarto artículo se estudiaron 13 casos de linfoma B cutáneo primario de la zona marginal procedentes de la Fundación Jiménez Díaz, que incluían casos consulta del Hospital de Guadalajara, el Hospital Universitario Insular Materno Infantil de Canarias, Hospital Clínico Lozano Blesa de Zaragoza, Hospital Universitario Puerta de Hierro de Majadahonda. Los criterios diagnósticos aplicados en todos los casos fueron revisados según la clasificación de los linfomas de la Organización Mundial de la Salud y la última

clasificación de la WHO-EORTC de linfomas cutáneos y los diagnósticos fueron revisados por dos patólogos (MAP y SMRP). El diseño de los estudios fue aprobado por el comité ético de cada Hospital. Para la realización de los estudios se siguieron los principios de la declaración de Helsinki.

TÉCNICAS BASADAS EN PROTEINAS

INMUNOHISTOQUÍMICA

Las tinciones de inmunohistoquímica se realizaron en los Servicios de Anatomía Patológica de la Fundación Jiménez Díaz y del HUMV. Se siguió el protocolo Envision FLEX de Dako (Glostrup, Dinamarca) sobre cortes de tejido parafinado de 4 mm de grosor, con un método de detección basado en avidina/biotina peroxidasa. Como cromógeno se empleo diaminobenzidina y se utilizó un imunoteñidor de Dako.

En el segundo artículo se emplearon los siguientes anticuerpos: ALK (ALK-1, RTU; Dako), CD3 (rabbit polyclonal, RTU; Dako), CD4 (4B12, RTU; Dako), CD8 (C8/144B, RTU; Dako), CD30 (Ber-H2, RTU; Dako), granzyme B (GRB7, 1/25, Dako), MUM1 (RTU, Dako), perforin (5B10, 1/10, Thermo Fisher Scientific), P-STAT3 (D3A7, 1/400 Cell Signaling), TIA1 (TA-1, 1/50, Abcam), P-STAT5 (D2A37, 1/200, Cell Signaling), TCR- βF1 (8A3, 1/40 dilution; Thermo Scientific), P63 (RTU, Dako), STAT3 (F-2, 1/100, Santa Cruz Biotechnology).

Los anticuerpos utilizados en el artículo 3, junto con los métodos de tinción se resumen en la tabla 2.

Tabla 2. Anticuerpos utilizados en el artículo 3

ANTICUERPO	CLON	MARCA	REFEREN -CIA	CON- TROL	RECUPERA- CION	AMPLIFI- CACION	DILUCIÓN/ INCUBACION TIEMPO	TEÑIDOR	TEMPERA- TURA
CD30-L	Ber-H2	DAKO	IR602	Tonsil	low	Flex+	RTU 4´	OMNIS	4º
CD20cy	L26	DAKO	GA604	Tonsil	High	Flex	RTU 20	OMNIS	4º
CD79	JBC117	DAKO	IR621	Tonsil	Low	Flex	RTU 8	OMNIS	4º
BCL2	124	DAKO	IR614	Tonsil	High	Flex+	RTU 20	OMNIS	4º
CKAE1-AE3	AE1/AE3	DAKO	GA053	Tonsil	High	Flex	RTU 10	OMNIS	4º
PAX5	DAK-Pax5	DAKO	IR650	Tonsil	High	flex+	RTU 15´	OMNIS	4º
MUM1	MUM1p	DAKO	IR644	Tonsil	High	flex +	RTU 30´	OMNIS	4º
CD15	Carb-3	DAKO	GA062	Tonsil	High	Flex	RTU 15	OMNIS	4º
CD 3-L	Policlonal	DAKO	GA503	Tonsil	Low	Flex+	RTU 10	OMNIS	4º
Ki67 -L	MIB-1	DAKO	GA506	Tonsil	low	Flex	RTU 20	OMNIS	4º
PD1	NAT105 C	CNIO		Tonsil	low	flex	1/500 20´	OMNIS	20º
PD-L1 (28.8)	28.8	DAKO	SK005	Tonsil				AUTOSTAINER Link 48	4º
HISTO-SONDA EBER	DNP probe	VENTANA	760-1209	EBER +	proteasa	Iviewblue ISH	RTU 28´	BENCHMARK	4º

En el cuarto artículo se emplearon los anticuerpos de DAKO siguientes, según los métodos indicados previamente para los anticuerpos de dicha casa comercial: CD30, CD15, CD20, CD3, PD1, κ , λ , IgG, IgM, IgD, IgA, CD123, Bcl6, Bcl2, p53, Pax5, CD21, CD23, CD5, Ki67, MYC, y pSTAT3.

El estudio de la presencia del virus de Epstein-Bar mediante hibridación in situ se hizo según el protocolo detallado en la tabla anterior utilizando la histosonda de la casa VENTANA.

ESTUDIO DEL REORDENAMIENTO DE 6P25.3 Y P63 MEDIANTE FISH O "HIBRIDACIÓN IN SITU MEDIANTE FLUORESCENCIA

Para estudiar el estado del locus 6p25.3 se realizaron estudios de FISH, empleando sondas comerciales de separación del locus IRF4-DUSP22 (6P25.3) break-apart probe (KBI-10613; kreatech, Leica, Spain), y DNA FISH de Empire dual color break apart TP63 (3q28), siguiendo los protocolos convencionales. Se realizó el contaje por cada preparación de 100 núcleos.

TÉCNICAS BASADAS EN EL DNA

Se extrajo DNA genómico de las muestras parafinadas utilizando el kit RecoverAll Multi-Sample RNA/DNA (Invitrogen, Carlsbad, CA) de acuerdo al protocolo establecido. El DNA se cuantificó con Qubit® dsDNA BRAssay kit (Invitrogen) .

Se llevó a cabo una reacción en cadena de la polimerasa (PCR) para analizar la posible expansión clonal de células B y T. El DNA se extrajo de cortes de parafina y la clonalidad se detectó con PCR para las recombinaciones del gen de los receptores de Inmunoglobulinas y TCR.[47] Se incluyeron controles positivos y negativos apropiados en todos los experimentos. La clonalidad se detectó siguiendo las recomendaciones establecidas.[48] Cada amplificación mediante PCR se realizó dos veces.

Para detectar la presencia de mutaciones en MYD88 utilizamos los primers descritos por Jiménez et al[49] y una PCR cuantitativa (qPCR) basada en la discriminación alélica. Las reacciones se realizaron por triplicado en el sistema Light Cycler 480 Real-Time PCR (Roche). Los ensayos se hicieron en placas de 384 pocillos en un volumen de reacción de 10 µ L utilizando 50 ng de DNA genómico (gDNA), 5 µ L de Light Cycler 480 Probes Master, 0.15 µ L primer (20 µ M, wild type o mutado),y 0.1 µ L de sonda (20 µ M). La muestra de reacción

fue incubada a 95º durante 10 minutos, seguido de 45 ciclos de amplificación a 95°C durante 10 segundos y 60°C durante 30 segundos. Los datos se analizaron con el software Light Cycler 480 SW 1.5 (Roche) utilizando el método Δ Cp. Los valores de Cp se analizan y muestran una SD de < 0.25. Los casos con Cp > 20 se consideran wild type.

En el artículo 4, en casos con DNA de buena calidad suficiente, se realizó un panel customizado de genes asociados con frecuencia a linfomas B de bajo grado. El panel incluía los siguientes genes diana: *TP53, ARID1A , BRAF , WHSC1 , PLCB1 ,SPEN , MEF2B , MYD88 , CCND3 , MAP2K1 , CCND1 ,MAP3K14 , NFKBIE , MLL2 , XPO1 , CHD2 , BTK , BIRC3 ,ATM , NOTCH1 , NOTCH2 , POT1 , CXCR4 , TNFAIP3 ,SF3B1 , KLF2 ,* y *TLR2.* La secuenciación de doble cadena elimina los falsos positivos en las mutaciones C-T que pueden aparecer por eventos de desaminación durante la fijación de la formalina. Las sondas para este panel customizado fueron diseñadas siguiendo el DesingStudio de Illumina y consisten en 1287 amplicones de un tamaño medio de 175pb y una región diana acumulada de 140 kb. Los polimorfismos se evitaron para el diseño de los primers. Cuando únicamente una cadena podía ser evaluada, solo validamos la mutación si su presencia en el mismo punto era detectada en otra muestra del mismo paciente.

El AQ10 Target enrichment se llevo a cabo en el DNA extraído de las muestras parafinadas de acuerdo al protocolo del fabricante (TruSeq Amplicon—Cancer Panel Library Preparation Guide; January 2017; Illumina). La cantidad total de DNA obtenido variaba entre 30 y 100 ng. Tras la preparación de las librerías, la indexación y la purificación con bolas magnéticas, las librerías (2 por muestra, 1 por cadena) se cuantificaron con Qubit® (Thermo Fisher Scientific),

se normalizaron con bolas magnéticas y se agrupan para la secuenciación. El pool de librerías fue secuenciado con Miseq Reagent Kit V2 (paired-end, 2Å~151) en MiSeq instrument (Illumina), según indica el protocolo del fabricante.

ANÁLISIS ESTADÍSTICO

En el segundo artículo la asociación entre subgrupos genéticos e inmunohistoquímicos con la supervivencia global (OS) y con la supervivencia libre de progresión se analizó mediante curvas de Kaplan-Meier. Las diferencias entre los pacientes de los diferentes subgrupos genéticos en cuanto a características clínicas y del fenotipo del tumor se analizarón mediante los test de $\chi 2$ y Wilcoxon rank-sum test.

En el cuarto artículo el análisis estadístico se realizó utilizando el test exacto de Fisher (2-tailed P-values) y el análisis de la varianza de un factor utilizando STATA, versión 14.2.

TRATAMIENTO DE LA BIBLIOGRAFÍA

La introducción de las citaciones en el texto se ha realizado mediante el programa gestor de referencias bibliográficas Endnote™ seleccionando el estilo de la “American Medical Association” de citación.

4. RESUMEN Y DISCUSIÓN DE LOS RESULTADOS

Los resultados obtenidos a través de los cuatro trabajos publicados permiten confirmar la hipótesis inicialmente establecida: **Una clasificación de los linfomas cutáneos con expresión de CD30 que integre datos clínicos, histológicos, inmunofenotípicos y moleculares permite precisar el diagnóstico y predecir mejor el pronóstico de los pacientes, así como seleccionar mejor los candidatos a terapias dirigidas.**

1. Los hallazgos moleculares son útiles para clasificar mejor los procesos linfoproliferativos primarios cutáneos CD30+ y pueden ser de ayuda para identificar pacientes que se beneficien de terapias dirigidas.
2. Los ALCL tanto cutáneos como sistémicos con reordenamiento de DUSP22 incluidos en nuestro trabajo tienen características morfológicas e inmunofenotípicas específicas como la presencia de marcadores T y la ausencia de marcadores citotóxicos, así como la ausencia de activación de la vía JAK/STAT que pueden ayudar a identificarlos.
3. La UMC-EBV+ incluye en nuestra serie, lesiones con patrón histológico polimorfo, difuso y angiocéntrico que aparecen en pacientes inmunocomprometidos y que comparten un pronóstico clínico favorable, no siendo necesario el uso de terapias agresivas para su resolución.
4. La presencia de un mayor porcentaje de células CD30+ con morfología Hodgkin-like en nuestra serie parece asociarse a la progresión

histológica de las lesiones y a la presencia de un mayor número de recidivas en nuestros pacientes.

ARTÍCULO 2

De los 31 casos en los que se analizó el reordenamiento de p63, 1 caso (1 de 31, 3,2%) fue positivo, 26 fueron negativos (26 de 31, 83,8%), y 3 mostraron ganancias de p63(3 de 31, 9,7%). Uno de los casos (1 de 22, 5,5%) tenía ganancia de DUSP22, y otro caso amplificación de DUSP22. Veinticinco casos (25 de 31, 80,6%) fueron clasificados como ALCL triple negativos, y 6 casos tenían reordenamientos de DUSP22, incluyendo 4 ALCLs ALK-(4 de 22, 18,2%) y 2 pcALCL (2 de 9, 22,2%), estos casos representan la cohorte del estudio.

La tabla 1 incluida en el artículo 2, muestra los datos clínicos y demográficos así como los datos inmunofenotípicos y de seguimiento de los pacientes con ALCL y reordenamiento de DUSP22.

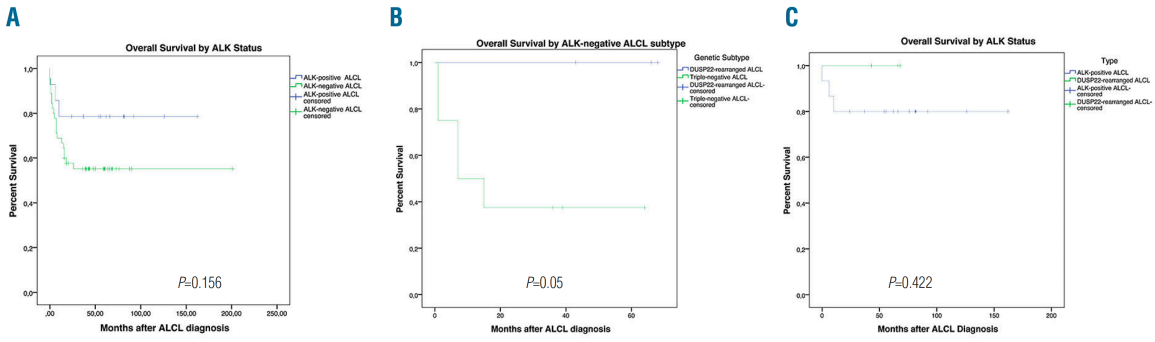

Figura 4. Supervivencia global de los pacientes en función del subtipo genético

Como muestra la figura 4, En consonancia con los resultados de publicaciones previas,[4] los pacientes con ALCLs ALK- tenían peor pronóstico en nuestra serie que los pacientes con ALCLs ALK+ (OS a 3 años 52% frente a 95%

con un CI: 36-68% vs 80-95%) CI:60-100%, log-rank, P=0,156). Los pacientes con ALCLs con reordenamiento de DUSP22 mostraban datos de OS mejores que los de los pacientes con ALCLs triple negativos (OS a 3 años 100% vs 28%, 96% CI:4-725;log-rank, P=0,05, para los pacientes triple negativos) y similar a la OS de los pacientes ALK+ (OS a 3 años: 80%, 96%CI: 60-100%; log-rank, P=0,422).

Como estaba descrito previamente[50], los ALCL con reordenamiento de DUSP22 muestran, en nuestros pacientes, características histológicas similares en todos los casos. En los casos de ALCLs, la arquitectura del LN estaba borrada por una infiltración neoplásica de células de tamaño intermedio, más pequeñas que las observadas en los ALCLs triple negativos y en los ALK+ con un patrón de crecimiento en sábana y una apariencia monomorfa. Estos hallazgos se repiten en todos nuestros casos. Las células neoplásicas muestran pseudo-inclusiones y nucléolos prominentes en las células “doughnut” aunque estos hallazgos no son específicos de este grupo. La presencia de células Hallmark, así como figuras mitóticas y cuerpos apoptóticos son frecuentes en nuestros casos. Predominan las células tumorales sin infiltrado linfohistiocitario ni microambiente inflamatorio prominente. No se observó invasión sinusoidal, a diferencia de lo que ocurre en los ALCLs ALK+. Los triple negativos tenían una morfología más variable con células Hallmark y grandes células polimorfas y multinucleadas.

Los dos casos de pcALCL tenían un patrón bifásico como el publicado previamente por Onaindia et al.[51] A pequeño aumento, se observaba una lesión nodular dérmica con un denso infiltrado linfoide y ulceración en superficie. El infiltrado neoplásico estaba compuesto de células atípicas de tamaño

intermedio-grande, con citoplasma granular abundante, entremezcladas con células Hallmark. Se observaba además un patrón epidérmico característico, similar al de la reticulosis pagetoide con linfocitos pequeños intraepidérmicos de características atípicas, con un núcleo hipercromático irregular. La presencia de figuras de mitosis y cuerpos apoptóticos era frecuente en el infiltrado dérmico y no había apenas PMN eosinófilos ni neutrófilos en el infiltrado.

En los ALCL con reordenamiento de DUSP22 las células tumorales expresaban al menos un marcador T, CD3 y/o la cadena beta del receptor T(TCRβF1), eran negativas para ALK y expresaban CD30 de forma difusa y fuerte. TCRβF1 no estaba disponible en el caso 2 pero CD3 era positivo. El caso 5 era CD3- pero TCRβF1 era positivo. Estos marcadores acentúan el patrón en sábana de los ALCLs y el patrón epidermotropo reticulosis pagetoide-like en los cutáneos. Todos los casos tenían un fenotipo no citotóxico. Los marcadores subrogados de la vía JAK/STAT (pSTAT1, pSTAT3 y pSTAT5) fueron negativos en los 6 casos con una expresión inferior al 20% de las células tumorales.

Con estos resultados podemos decir que los datos publicados en nuestro artículo sobre la morfología de los ALCL con reordenamiento de DUSP22, en los que se muestran células intermedias con morfología tipo doughnut y anundantes células Hallmark con figuras de mitosis y apoptosis son concluyentes con los publicados hasta ahora en la literatura.[50] Además, nuestros casos de pcALCL tienen la morfología bifásica descrita previamente en los pcALCL con DUSP22 translocado y en los casos de LyP con esta translocación.[51,52] Por último, como han indicado otros grupos, nuestros casos presentan ausencia de activación de la vía de JAK/STAT a diferencia de lo que se creía en un principio sobre que los ALCL tanto ALK- como ALK+ tienen activación de la vía de JAK/STAT.[40]

ARTÍCULO 3

Las características clínicas, histológicas e inmunofenotipicas de los casos de UMC-EBV+ se resumen en tablas 4,5 y 6.

En primer lugar, como refleja la tabla 4, nuestra serie muestra la gran variabilidad clínica de la UMC-EBV+. Expandimos el espectro clínico de estas lesiones describiendo por primera vez su presentación como múltiples úlceras genitales. En los casos descritos con anterioridad la presentación clínica como úlcera solitaria era la más frecuente, con tan sólo un 17% de los casos siendo multifocales.[53] 5 de nuestros 9 casos son multifocales con localizaciones concordantes con las publicadas con mayor frecuencia en la literatura: orofaringea seguida de piel y mucosa digestiva.[53]

En segundo lugar, desde el punto de vista histológico, consideramos que el reconocimiento en nuestra serie de un espectro de lesiones que va desde un patrón polimorfo a uno más difuso (“large B-cell lymphoma-like”), propuesto por primera vez por Rausch y colaboradores[54], debería tenerse en cuenta en futuras revisiones de la clasificación de los procesos linfoproliferativos EBV relacionados. Revisando la literatura nos encontramos con que tradicionalmente los casos localizados en mucosa intestinal y en la cavidad oral tienen un patrón más polimorfo con presencia de células Reed-Stengberg y Hodgkin-like y un importante microambiente inflamatorio compuesto por histiocitos, PMN, células plasmáticas y linfocitos T CD3+ dispuestos en banda delimitando la lesión.[55]

Tabla 4. Características clínicas y seguimiento de la serie de pacientes con UMC-EBV+

NÚMERO DE CASO	EDAD/ SEXO	LOCALIZACIÓN	PRESENTACIÓN CLÍNICA	MORFOLOGÍA CLÍNICA	INMUNOSUPRESIÓN ASOCIADA	TRATAMIENTO Y EVOLUCIÓN	CUANTIFICACIÓN DNA EBV
1	55/M	Amígdala, cavum, orofaringe, hipofaringe	Sangrado orofaringeo grave	Múltiples Úlceras	HIV	Resolución con 4 Ciclos de CHOP	Baja (84 copias/mL)
2	57/F	Recto bajo	Sangrado rectal	Eritema inespecífico sin úlceras ni erosiones	EII	Cese del sangrado Todavía lesiones en última colonoscopia	NP
3	70/M	Recto	Sangrado rectal	Lesión tumoral	HIV	Resolución espontánea	NP
4	74/M	Tuberosidad maxilar izquierda, paladar, amígdala lingual izquierda	Lesiones orales múltiples	2 úllceras	Methotrexate (RA)	Resolución tras la suspension del fármaco	NP
5	87/F	Muslo	Asintomático, multiples lesiones cutáneas	Nódulos cutáneos violáceos	MDS tipo MMCL	Muerte por ICC	NP
6	75/F	Región mandibular izquierda, gingival	Lesión oral dolorosa	Tumor ulcerado	Corticoides orales y methotrexate (RA)	Resolución tras la suspension del fármaco	NP
7	87/F	Suelo de la boca	Lesión oral dolorosa	Tumor ulcerado	Senescencia	Resolución tras RT	NP
8	87/F	Piel de la zona de la cadera	Lesión cutánea asintomática	Placa violácea infiltrada (Figura 1)	Senescencia	Resolución tras tratamiento con bendamustina y Rituximab	NP
9	74/M	Glande y prepucio	Múltiples brotes de lesiones	Multiples pápulas y erosions	Senescencia	Resolución espontánea	NP

M: hombre, F: Mujer, MDS: Síndrome mielodisplásico, RT: Radioterapia, MMCL: Leucemia crónica mielomonocítica, RA: Artritis reumatoide, EII: Enfermedad inflamatoria intestinal, HIV: Virus de la inmunodeficiencia humana

Tabla 5. Principales características histológicas de los casos

CASO	ULCERACIÓN/ NECROSIS	CÉLULAS REED-STERNBERG-LIKE	INFILTRADO PERIVASCULAR	ANGIOTROPISMO	PLASMA-TICAS	HISTIOCIT0S	PMN	INFILTRA-DOS T EN MÁRGENES	C-EPITELIALES EBV+	OTRAS BIOPSIAS CON C-EBV+	PATRÓN HISTOLÓGICO
1	P	P	P	A	P	P	P	P	A	Gastritis C- EBV+ Medulla osea C-EBV+	Polimorfo
2	P F	P	P	P	P, Policlonales	P	P	P	A	A	Polimorfo
3	P	P	P	A	P	P	P	P	A	A	Polimorfo
4	P	P	P	A	P	P	A	P	P	A	Polimorfo
5	P	P	P	P	A	A	A	P	A	A	Difuso
6	P	P	P	A	S	S	S	P	A	A	Difuso
7	P	P	P	A	P	P	P	P	A	A	Polimorfo
8	P	P	P	P	S	P	P	P	A	A	Difuso
9	P	P	P	P	P	P	P	A	A	A	Polimorfo

P: Presente, A: Ausente, F: Focal, C-: Células

Tabla 6. Características inmunofenotipicas de las células grandes tumorales CD30+

CASO	CD79a	CD20	CD30	Bcl-2	AE1-AE3	PAX5	MUM1	CD15	MIB1-Ki67	CD3	PDL1	PD1	EBER	PAX5/PDL1
1	+	-	+	+	NP	+	+	-	+	-	+	-(+acom)	+	+
2	NP	+	+	NP	NP	NP	NP	NP	NP	-	NP	NP	+	NP
3	+	+	+	-	NP	+	+	+	+	-	+	-(+acom)	+	+
4	+	+	+	-	-	+	+	+	+	-	+	-(+acom)	+	+
5	+	+	+	+	NP	NP	+	NP	+	-	NP	NP	+	NP
6	NP	+	+	+	NP	NP	NP	NP	+	-	NP	NP	+	NP
7	NP	-	+	-	-	+	+	-	+	-	NP	NP	+	NP
8	+	+	+	-	NP	+	+	NP	+	-			+	NP
9	+	-	+F	-	-	+W	+	-	+	-			+	NP

NP: No realizado. Acom: Células T reactivas acompañantes. W: Débil, F: Focal

El contexto de inmunosupresión de nuestros pacientes es similar al descrito hasta la fecha en la literatura e incluye la edad, los fármacos inmunosupresores, los tumores, las enfermedades autoinmunes y recientemente, las inmunodeficiencias primarias y los pacientes VIH+.[56] Las características clínicas e histológicas son similares en todos los contextos.

Por último, en nuestros casos hemos hallado otros dos hechos no descritos anteriormente en la literatura. En primer lugar, la expresión por células tumorales en los 3 casos en los que pudo realizarse de PDL1, comprobado mediante doble tinción Pax5/PDL1 (ver figura 4 del artículo 3). Daroontum y colaboradores defendían que en las UMC-EBV+ el PDL1 es expresado por los macrófagos del micoambiente tumoral mientras que las células tumorales eran negativas, a diferencia de lo que ocurre en el DLBCL EBV+, pero en nuestros casos las células tumorales también expresan PDL1.[57] Además, nuestro artículo es el primero que encuentra células EBER positivas en el epitelio adyacente a la UMC-EBV+. Este hallazgo había sido descrito en carcinoma nasofaríngeo

indiferenciado, así como en la leucoplasia vellosa y en células epiteliales de encías en periodontitis.[58,59]

ARTÍCULO 4

Las características clínicas, histológicas, inmunofenotípicas y moleculares halladas en los 13 casos de nuestra serie se resumen en las tablas 7,8,9 y 10.

La presencia de células grandes aisladas en los casos de PCMZL ha sido descrita en otras series en la literatura.[60-62] y algunos de estos estudios ya mencionaban la expresión de CD30 por algunas de estás células grandes.[63]

Desde entonces pocos trabajos han estudiado la presencia de estas células en los PCMZL. Rodríguez-Pinilla y colaboradores describieron la presencia de células grandes CD30+ rodeadas por células T PD1+ en PCMZL.[64] En nuestra serie, además de células CD30 en los folículos reactivos, encontramos una distribución más difusa de estas células CD30+ fuera de éstos y también rodeadas de células T, algunas de morfología atípica con expresión de PD1. Como ejemplificamos en los casos 3 y 11 de nuestra serie, un incremento en el porcentaje de estas células CD30+ podría asociarse con progresión histológica y un mayor número de recidivas.

La presencia de rosetas de células T PD1+ encontradas en la mayoría de nuestros casos, podría estar relacionada con la presencia de picos clonales T mediante técnicas de PCR de TCR-gamma y de TCR-beta. Estos picos T habían sido descritos previamente tanto en PCMZL[65] como en otros linfomas B.[66] La expansión clonal de estas células T PD1+ que rodean las células CD30 podría explicar este hallazgo.

En ocasiones estás células CD30+ con morfología Hodgkin-like han conducido en algunos casos al diagnóstico erróneo de linfoma de Hodgkin cutáneo primario. Desde nuestro punto de vista este diagnóstico es erróneo y los casos descritos como esta entidad corresponden en muchas ocasiones a otros linfomas cutáneos con células CD30+ con morfología Hodgkin-like como LyP, pcALCL o como en nuestros casos PCMZL.

El cambio de clase de cadenas ligeras observado en nuestro caso número 12 es un evento muy inusual en estos linfomas cutáneos de la zona marginal. Sólo ha sido descrito de forma ocasional en otros linfomas como el linfoma folicular[67,68] o la enfermedad linfoproliferativa post-transplante[69] y se ha relacionado en estos casos con progresión de la enfermedad y transformación.

Los casos descritos aquí tienen ciertas similitudes con los descritos previamente como linfoma marginal tipo blástico[70] o transformación de un PCMZL a un linfoma B difuso de células grandes. Sin embargo, nuestros casos muestran una morfología compatible con PCMZL con únicamente un incremento parcial en el número de células grandes, pero no la morfología de un DLBCL. Además, en esos estudios no se analiza la expresión de CD30.

Existen pocas series en la literatura donde el número de pacientes seguidos permita estimar de forma precisa la tasa de recurrencias y la supervivencia libre de enfermedad en estos pacientes.[62,71,72]

Una de las series de PCZML con mayor número de pacientes publicada por Servitje y colaboradores[71] incluye 137 casos en los cuales concluyen que un estadio T3 y las lesiones multifocales son las características que más se relacionan clínicamente con un mayor número de recidivas y una supervivencia libre de enfermedad acortada. Estos pacientes descritos en su serie tienen

características clínicas similares a los nuestros con predominio de hombres y localización en tronco y extremidades más frecuente.

Como nosotros, ellos también utilizan el sistema de estadificación de EORTC/ISCK, pero a diferencia de nuestra serie, muchos de sus pacientes (51%) se encuentran en estadios T1 (T1a, T1b) y el 44% presenta recidivas.[71] En nuestros casos, que se han seleccionado en base al porcentaje de células grandes CD30+, el porcentaje de recaídas en mayor (69%) y únicamente un 31% de los casos estaban en un estadio T1. Nuestra serie muestra una relación estadísticamente significativa entre la presencia de un porcentaje mayor de células CD30+ y la progresión clínica de la enfermedad entendida como estadios clínicos más avanzados y mayor número de recaídas.

Tabla 7. Principales características clínicas y de seguimiento de los pacientes

CASO	EDAD (LR)	SEXO	Nº DE LESIONES (LR)	LOCALIZACIÓN	EORTC/ISCL ESTADIO	RECAÍDAS CUTÁNEAS	RECAÍDAS EXTRACUTÁNEAS	OTROS LINFOMAS	TRATAMIENTO	SEGUIMIENTO
1	44	M	1	Brazo derecho	T1a	No	No	No	Cirugía	3 meses
2	51	M	1	Mejilla	T2	Si, 2	No	No	Cirugía	3 años
3	71	F	3 (masas)	Mama/pectoral Cadera Muslo	T3b	Si, 3	No	No	R-CHOP(x4)	11 años
4	82	F	3	Ambos hombros	T2c	Si, 2	No	No	Cirugía	3 años
5	59	F	1	Glúteos	T1a	No	No	No	Cirugía	1 año
6	37	M	1	Espalda	T1a	No	No	No	Cirugía	3 años
7	54	M	1	Espalda	T1a	No	No	No	Cirugía	1 año
8	35	M	6	Coxis Muslo derecho Pierna izquierda Pie derecho(2) Tobillo izquierdo	T2c	Si, 3	No	No	Rt, rituximab	5 años
9	75	M	2	Escápula izquierda	T2a	Si, 2	No	Si, linfoma B difuso de células grandes testicular (2011)	Cirugía	3 meses
10	72	M	>10	Espalda	T2c	Si, multiple >4	No	No	Cirugía Rituximab intralesional	3 años
11	72	M	3	Brazo izquierdo	T2c	Si, multiple>10	No	No	Cirugía CE intralesional RTX intralesional RT	24 años
12	68	M	4	Muslo derecho Espalda	T2c	Si, 4	No	No	Cirugía	3 años
13	39	M	3	Brazo izquierdo	T2b	Si, 3	No	No	CE intralesional CE tópicos	15 meses

LR: última recaída CE: Corticoesteroides, RTX: Rituximab

Tabla 8. Características histológicas

CASO	PATRÓN HISTOLÓGICO	INFILTRADO TUMORAL	FOLICULOS REACTIVOS	INFILTRADO ACOMPAÑANTE	PRESENCIA DE CÉULAS GRANDES(%)	CÉLULAS CD30+ DISTRIBUCIÓN Y %	CÉLULAS REED-STERNBERG O,HODGKIN-LIKE	EPITELIO-TROPISMO
1	N	Células B y plasmáticas	P	T	10-15	10, I	P	A
2	N	Células B y plasmáticas	P	T	10-15	10, I	P	A
3	D	Células B	A*	T	40-50	40, D	P	A
4	N	Células B	P	T Rosetas en células CD30	30	30, I, D	P	A
5	N	Células B y plasmáticas	P	T Rosetas en células CD30	20-30	10, I	P	A
6	N	Células B y plasmáticas	P	T Rosetas en células CD30	20	10, I	P	A
7	N	Células B y plasmáticas	P	T Rosetas en células CD30	10	10, I	P	A
8	N	Células B y plasmáticas	P	T Rosetas en células CD30	20	15, I	P	A
9	N	Células B y plasmáticas	P	T	20	15, I	P	A
10	N, M	Células B y plasmáticas	A*	T Rosetas en células CD30	10	10, I	P	A
11	D	Células B	A*	T Rosetas en células CD30	30	15, I, D	P	A
12	N	Células B y plasmáticas	P	T Rosetas en células CD30	20	15, I	P	A
13	N	Células B y plasmáticas	P	T Rosetas en células CD30	30	30, I, D	P	A

N: Nodular, D: Difuso, I: Intersticial, M: Mixto, A: Ausente, A*: Ausente con folículos distorsionados, P: Presente

Tabla 9. Características inmunofenotípicas

CASO	CD20	CD30	CD15	Pax5	EBER	p53	Ki67	Bcl6	MYC	Bcl2	pSTAT3	CÉLULAS CD123	Restricción de cadenas ligeras	Cadena pesada dominante
1	+	+ en células grandes	-	+	-	-	20%	-	+ en S células grandes	+	-	PC	LAMDA	IgG
2	+	+	-	+	-	-	20%	-	+ en S células grandes	+	S	S	KAPPA	IgM
3	+	+	-	+	-	+ S	50%	-	NP	+	NP	NP	KAPPA	IgM
4	+	+	-	+	-	-	20-30%	-	+ en S células grandes	+	-	PC	KAPPA	IgG-IgG4
5	+	+	-	+	-	-	20-30	-	+ en S células grandes	+	-	PC	NO	No plasma cells
6	+	+	-	+	-	-	20%	-	+ en S células grandes	+	-	C Alrededor de vasos	LAMBDA	IgM
7	+	+	-	+	-	-	20%	-	+ en S células grandes	+	-	C Alrededor de vasos	KAPPA	IgM (Myd88 not mutated)
8	+	+	-	+	-	-	30%	-	+ en S células grandes	+	-	C Alrededor de vasos	KAPPA	IgG
9	+	+	-	+	-	-	20%	-	+ en S células grandes	+	-	C Alrededor de vasos	KAPPA	IgG4 Myd88 mutated
10	+	+	-	+	-	-	30%	-	+ en S células grandes	+	-	C Alrededor de vasos	LAMBDA	IgG
11	+	+	+	+	-	+ S	30%	-	+ en S células grandes	+	-	C Alrededor de vasos	NO	No presenta células plasmáticas
12	+	+	-	+	-	-	30%	-	+ en S células grandes	+	-	C Alrededor de vasos	LAMBDA	IgG
13	+	+	-	+	-	-	30%	-	+ en S células grandes en folículos	+	-	A	KAPPA	IgG

S: Aisladas NP: No realizado, PC: Presente en acúmulos, C: Acúmulos, A: Ausente

Tabla 10. Características géneticas y moleculares.

CASO	TCR gamma Reordenamiento	TCR beta Reordenamiento	IgH reordenamiento	Kappa, kappa del, lambda reordenamiento	MYD88 L265P Mutación	MALT1 translocación	Otras mutaciones
1	Clonal	Clonal	FR1, FR2, FR3 Polyclonal	Ig kappa and kappa del clonal Ig lambda polyclonal	NP	NP	*NOTCH2-A3F*
2	No Clonal	Clonal	FR3 clonal	Ig kappa clonal	NP	NP	NE
3	No clonal	No clonal	FR1, FR2, FR3 clonal	NP	A	NE	*KMT2D-R5048L KMT2D-C349W*
4	Clonal	Clonal	FR1 clonal?	Ig kappa clonal Ig lambda and kappa del irregular polyclonal	A	A	WT
5	No clonal	No clonal	FR1,FR3 clonal FR2 pseudoclonal	Ig lambda clonal	NP	NP	NE
6	NP	NP	NP	NP	NP	NP	WT
7	NE	NE	No clonal	Ig kappa clonal	A	NP	NE
8	Clonal	Clonal	Pseudoclonal FR1 Clonal FR2 Polyclonal FR3	Kappa oligoclonal Lambda pseudoclonal Kappa del clonal	NP	NP	WT
9	Clonal	Clonal	FR1, FR2, FR3 clonal	NP	P	NP	*NFKBIE*
10	NE	NE	FR1 clonal FR2, FR3 polyclonal	NP	NP	NP	NE
11	No clonal	Clonal	FR2 clonal FR3 polyclonal	NP	NP	NP	*NOTCH2-A3F*
12	No clonal	Clonal	FR1 and FR2 clonal FR3 polyclonal	NP	NP	NP	NE
13	NE	NE	No clonal	Ig kappa clonal	NP	NP	NE

NP: No realizado, NE: No evaluable, WT: Wild type

Los estudios moleculares realizados en nuestros casos revelan mutaciones en los genes *KMT2D, NOTCH2, NFKBIE y MYD88*. Estas mutaciones han sido encontradas en otros estudios de linfomas marginales, particularmente en los localizados en anejos oculares[73] y en los linfomas marginales esplénicos[74] con algunos casos IgM positivos presentando mutaciones de *MYD88*.[75]

5. CONCLUSIONES

Conclusión general:

La expresión de CD30 puede verse en linfomas cutáneos tanto B como T. Aunque su expresión no es un marcador pronóstico universal, si que implica la posibilidad de utilizar terapia dirigida con BV.

Conclusiones específicas:

ARTÍCULO 1:

1. Los estudios moleculares de los casos de pcALCL y LyP revelan hallazgos heterogéneos que parecen relacionarse en algunos casos con hallazgos histológicos e inmunohistoquímicos.
2. Actualmente, a nivel molecular, proponemos clasificar los pcALCL en varios grupos: los ALK+, los ALK- con DUSP22 translocado, los ALK- con TP63 translocado, los triple negativos (ALK-, DUSP22-, TP63-) que son el grupo más frecuente y por último los que presentan el gen de fusión NPM1-TYK2 y la activación oncogénica de la vía de STAT3.
3. Existen alteraciones epigenéticas y en la via de NOTCH presentes en los pcALCL que pueden utilizarse potencialmente como dianas terapéuticas en pacientes refractarios a los tratamientos convencionales, ya que existen fármacos que actúan a ese nivel.

ARTÍCULO 2:

1. La presencia de acúmulos de células grandes o intermedias con una citología relativamente monoforma incluyendo células “Hallmark” y “doughnut” sin expresión de marcadores citotóxicos, podría ser un

marcador útil de la necesidad de realizar estudio FISH de DUSP22 en linfomas anaplásicos sistémicos.

2. En los pcALCL la presencia del patrón previamente descrito como “bifásico” en la histología es un buen indicador de la posible presencia de la translocación de DUSP22.
3. La misma translocación en el locus de 6p25 está descrita también en papulosis linfomatoide, sugiriendo un buen pronóstico en los casos en los que está presente, tanto en los cutáneos como en los sistémicos.
4. La expresión de marcadores T, así como la ausencia de marcadores citotóxicos y marcadores de activación de la vía de STAT parecen ser indicativos de la presencia de la translocación de DUSP22.

ARTÍCULO 3:

1. Es esencial definir el espectro clínico e histológico de la UMC-EBV+, así como identificar los factores que condicionan su evolución y necesidad de tratamiento para poder facilitar un manejo más adecuado de estos pacientes, teniendo en cuenta que muchos son ancianos e inmunodeprimidos con mayor riesgo de iatrogenia.
2. La mucosa genital es una localización posible de la UMC-EBV+.
3. Algunos pacientes diagnosticados previamente de linfoma B difuso de células grandes EBV+, NOS, han sido reclasificados como UMC-EBV+ teniendo en cuenta todas las características clínicas y su evolución, lo que implica que casos que previamente estaban clasificados como linfoma de células grandes y siguieron un curso indolente podrían en realidad ser UMC-EBV+.

4. El papel de la expresión de PDL1 en células tumorales y del microambiente, así como la relevancia de la presencia de células epiteliales EBER positivas en la proximidad de la lesión e implicación de la cuantificación del DNA de EBV en sangre periférica de estos pacientes con UMC-EBV+ deben ser estudiados en el futuro.

ARTÍCULO 4

1. La presencia de células neoplásicas grandes CD30+ en linfomas cutáneos primarios de la zona marginal no es infrecuente, y suele estar acompañada de rosetas de células T PD1+ en muchas ocasiones que pueden ser numerosas y con morfología atípica e influir en la presencia de picos clonales T en estos linfomas B.
2. Los casos con mayor porcentaje de células grandes CD30+ parecen tener un comportamiento más agresivo, con mayor número de recurrencias y masas tumorales mayores.
3. Por estos motivos expuestos en las conclusiones 1 y 2, consideramos que, en los casos de linfomas primarios cutáneos de la zona marginal con presencia de células Hodgkin-like debería añadirse el CD30 entre los marcadores inmunohistoquímicos realizados.
4. Debe tenerse en cuenta la posible presencia de estas células CD30+, Hodgkin-like en los casos de linfomas cutáneos de la zona marginal para realizar un correcto diagnóstico diferencial con la afectación cutánea por un linfoma de Hodgkin, ya que el pronóstico y el tratamiento es muy diferente en estos casos.

6. PERSPECTIVAS

Tras la realización de este trabajo quedan muchos campos abiertos en el estudio de la biología molecular y las alteraciones genéticas y epigenéticas en los linfomas cutáneos con expresión de CD30 que permitan clasificar mejor a los pacientes en función de su pronóstico y seguir identificando mejor a los candidatos a terapias dirigidas.

En el caso del ALCL, sería de gran ayuda identificar cuales son las vías que se activan de forma diferencial en ALCLs triple negativos, frente a los pcALCL triple negativos que hacen que los primeros debuten con estadios avanzados y mal pronóstico frente a los cutáneos que con una histología y fenotipo similar tienen un comportamiento clínico mucho menos agresivo.

Sería interesante identificar marcadores fenotípicos y moleculares, que además de la clínica, nos ayuden a diferenciar con facilidad pacientes con UMC-EBV+ con patrón difuso de pacientes con otros linfomas EBV+ más agresivos. Además de estudiar más en profundidad el significado de la presencia de EBV en el epitelio próximo a las lesiones de UMC-EBV+ y la importancia de la expresión de PDL1 en las células tumorales y del microambiente de estos pacientes.

En cuanto a los PCMZL, se podría ampliar el estudio y validarlo en otras poblaciones realizando un estudio de la expresión de CD30 en un numero amplio de pacientes con PCMZL y ver si los pacientes con un porcentaje mayor de células CD30+ presentan a lo largo de la evolución un mayor numero de recidivas y comprobar que como ocurre en nuestra serie, en los casos con progresión histológica aumenta el porcentaje de células grandes CD30+. Además, podría intentar validarse un modelo predictivo de

recidivas en estos pacientes que incluyese variables clínicas, fenotípicas y moleculares, así como estudiar en profundidad la presencia de clones T y su relación con rosetas o células PD1+ o la atipia de los infiltrados T.

CUARTA PARTE

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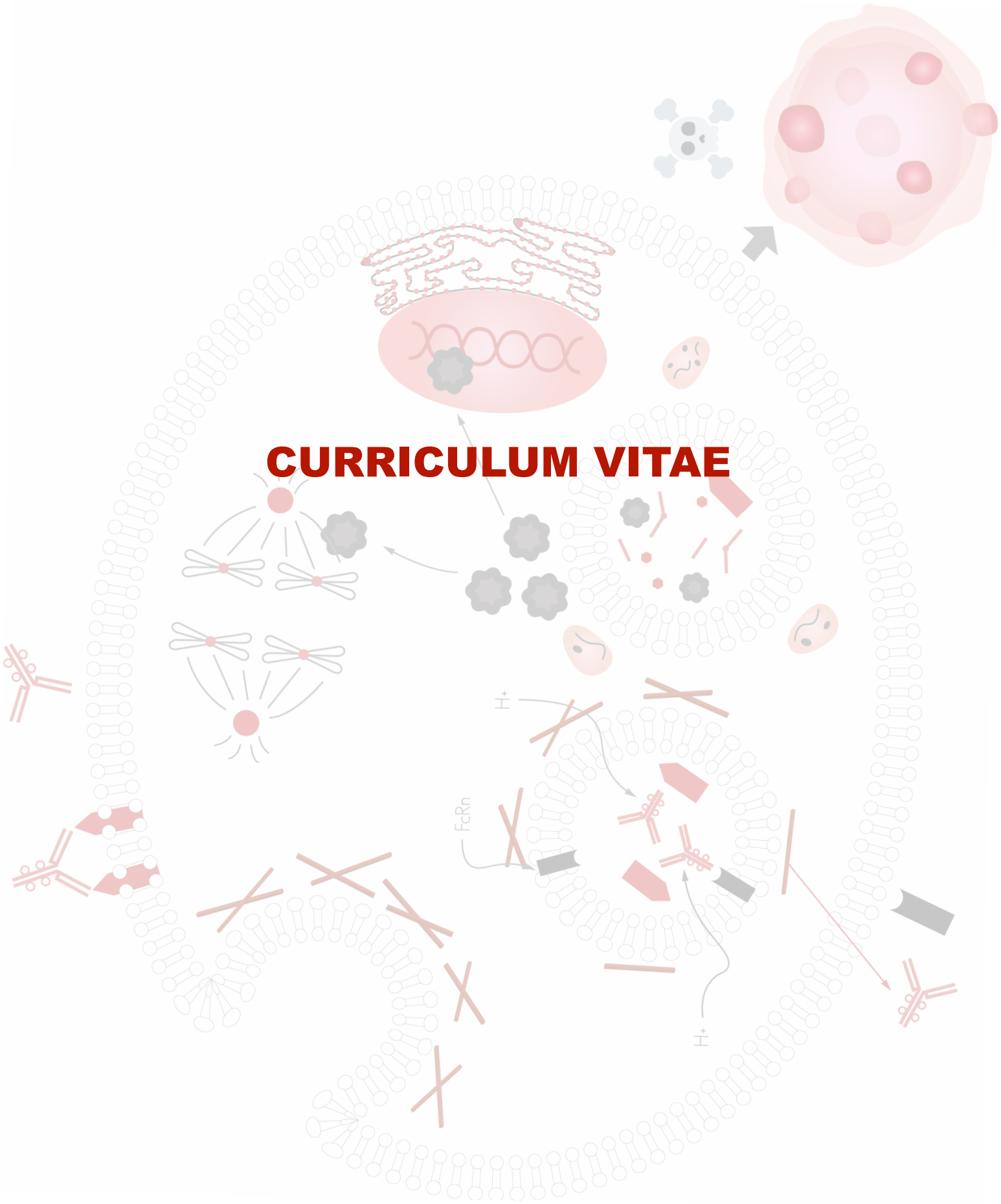
ANEXO I



CURRICULUM VITAE

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GOBIERNO DE ESPAÑA
MINISTERIO DE CIENCIA, INNOVACIÓN Y UNIVERSIDADES

FECYT FUNDACIÓN ESPAÑOLA PARA LA CIENCIA Y LA TECNOLOGÍA

Resumen libre del currículum

Descripción breve de la trayectoria científica, los principales logros científico-técnicos obtenidos, los intereses y objetivos científico-técnicos a medio/largo plazo de la línea de investigación. Incluye también otros aspectos o peculiaridades importantes.

Oposiciones y concursos:

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Obtención de plaza como Personal Estatutario Fijo del Servicio Aragonés de Salud, diciembre 2018. Convocatoria BOA del 20 de abril de 2017

Premios y becas

"En busca del fibroblasto perdido: identificación de nuevos marcadores de los fibroblastos asociados a cáncer epitelial". 44 Congreso Nacional de Dermatología y Venereología, celebrado en Zaragoza del 1 al 4 de junio de 2016. Premio Prof Gracía Perez a la mejor comunicación oral.

"Modelo predictivo de metástasis en ganglio centinela. teniendo en cuenta las variables histológicas clásicas y los nuevos parámetros de linfangiogénesis". X Reunión Nacional de Residentes de Dermatología. Valencia 25-26 de septiembre de 2015. Tercer premio a la mejor comunicación oral.

Beca de la Sección Vasco Navarra Aragonesa Riojana para el proyecto de investigación Microbioma en las placas de psoriasis. Reunión de la sección vasco-navarra-aragonesa-riojana de la AEDV. Logroño, 4 de octubre de 2014

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Estancias formativas:

- Durante los meses de octubre, noviembre y diciembre de 2014, rotación de Dermatopatología con el Dr Luis Requena Caballero en la Fundación Jimenez Diaz (Madrid)

- Durante los meses de abril, mayo y junio de 2016 rotación de Dermatología pediátrica con el Dr Antonio Torrelo en Hospital Niño Jesús de Madrid
- Durante los meses de enero y febrero de 2017 rotación en Dermatopatología y Linfomas cutáneos con el Dr Lorenzo Cerroni en Graz, Austria

Lucia Prieto Torres

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Situación profesional actual

Entidad empleadora: Hospital Clínico Universitario Lozano Blesa
Tipo de entidad: Instituciones Sanitarias
Categoría profesional: FEA Dermatología
Fecha de inicio: 03/06/2019
Modalidad de contrato: Estatuario/a

Entidad empleadora: Hospital San Jorge
Tipo de entidad: Instituciones Sanitarias
Categoría profesional: FEA DERMATOLOGÍA
Fecha de inicio: 01/02/2019
Modalidad de contrato: Estatuario/a
Régimen de dedicación: Tiempo completo

Entidad empleadora: Fundación Jiménez Díaz
Tipo de entidad: Fundación
Categoría profesional: Contrato Rio Hortega
Fecha de inicio: 29/01/2018
Modalidad de contrato: Becario/a (pre o posdoctoral, otros)

Entidad empleadora: Hospital de Tudela
Categoría profesional: FEA Dermatología
Fecha de inicio: 30/10/2017
Modalidad de contrato: Contrato laboral indefinido
Régimen de dedicación: Tiempo completo

Entidad empleadora: Hospital Clínico Universitario Lozano Blesa
Tipo de entidad: Instituciones Sanitarias

Departamento: Dermatología, Facultad de Medicina Universidad de Zaragoza
Categoría profesional: MIR 4 DERMATOLOGÍA
Fecha de inicio: 21/05/2013
Modalidad de contrato: FORMACIÓN SANITARIA ESPECIALIZADA
Funciones desempeñadas: Consultas Externas Quirófano Guardias. - Durante el primer año de Residente realización de 5-6 guardias mensuales de 24 horas, de presencia física en el Servicio de Urgencias, Hospital Clínico Universitario de Zaragoza. - Durante el segundo, tercer y cuarto año de Residente realización de 5-7 guardias mensuales de 24 horas de presencia física en la especialidad de Dermatología.

Formación académica recibida

Titulación universitaria

Estudios de 1º y 2º ciclo, y antiguos ciclos (Licenciados, Diplomados, Ingenieros Superiores, Ingenieros Técnicos, Arquitectos)

1 **Titulación universitaria:** Titulado Superior
Nombre del título: Máster Universitario en Dermatología Oncológica
Entidad de titulación: FUNDACION UNIVERSIDAD-EMPRESA. VALENCIA
Fecha de titulación: 15/12/2016

2 **Titulación universitaria:** Titulado Superior
Nombre del título: Máster Universitario en iniciación a la investigación en medicina
Entidad de titulación: Universidad de Zaragoza **Tipo de entidad:** Universidad
Fecha de titulación: 15/07/2014

3 **Titulación universitaria:** Titulado Superior
Nombre del título: Licenciado en Medicina y Cirugía
Entidad de titulación: Universitat de València **Tipo de entidad:** Universidad
Fecha de titulación: 27/08/2012

4 **Titulación universitaria:** Titulado Superior
Nombre del título: Máster de estudios en Metodología de la Investigación: Diseño y estadística en ciencias de la salud.
Entidad de titulación: Universitat Autònoma de Barcelona **Tipo de entidad:** Universidad

Doctorados

Programa de doctorado: Programa Oficial de Doctorado en Medicina
Entidad de titulación: Facultad de Medicina Universidad de Zaragoza **Tipo de entidad:** Universidad

Conocimiento de idiomas

Idioma	Comprensión auditiva	Comprensión de lectura	Interacción oral	Expresión oral	Expresión escrita
Alemán	A2	A2	A2	A2	A2
Inglés	C1	C1	C1	C1	C1

Experiencia científica y tecnológica

Actividad científica o tecnológica

Proyectos de I+D+i financiados en convocatorias competitivas de Administraciones o entidades públicas y privadas

1 **Nombre del proyecto:** RETROBIOMA Y MICROBIOMA EN LAS PLACAS DE PSORIASIS. PERTENENCIA AL GRUPO PSORIASIS DEL IIS

Entidad de realización: Instituto Aragonés de Ciencias de la Salud

Tipo de entidad: Entidad Gestora del Sistema Nacional de Salud

Nombres investigadores principales (IP, Co-IP,...): LUCIA PRIETO TORRES; CLAUDIA CONEJERO DEL MAZO; MARIANO ARA MARTIN

Nº de investigadores/as: 3

Fecha de inicio: 15/01/2015

2 **Nombre del proyecto:** SKIN SCC GENOMIC STUDY

Entidad de realización: COORDINADO POR LA UNIVERSIDAD LIBRE DE BRUSELAS

Nombres investigadores principales (IP, Co-IP,...): LUCIA PRIETO TORRES; IEVGENIA PASTUSHENKO; CEDRIC BANPLAIN

Fecha de inicio: 15/01/2015

Contratos, convenios o proyectos de I+D+i no competitivos con Administraciones o entidades públicas o privadas

Nombre del proyecto: OPTIMISE. Protocolo CAIN457A3302.ENSAYO CLÍNICO NOVARTIS SECUKINUMAB (COSENTYX)

Nombres investigadores principales (IP, Co-IP,...): LUCIA PRIETO TORRES; CLAUDIA CONEJERO DEL MAZO; MARIA ANTONIA CONCELLON DOÑATE; ANA LUISA MORALES MOYA; MARIANO ARA MARTIN

Fecha de inicio: 09/01/2015

Duración: 2 años

Actividades científicas y tecnológicas

Producción científica

Publicaciones, documentos científicos y técnicos

1 Lucía Prieto Torres; Álvaro Rivera Rodríguez; Mariano Ara Martín. Exploración dermatológica. Semiología básica y procedimientos comunes en urgencias pediátricas. pp. 117 - 125. Ergon, 2017. ISBN 978-84-16732-55-5
Tipo de producción: Capítulo de libro

2 Yolanda Gilaberte; Lucía Prieto-Torres; Ievgenia Pastushenko; Angeles Juarranz. Anatomy and function of the skin. Nanoscience in Dermatology. pp. 1 - 14. Elsevier, 2016. ISBN 978-0-12-802926-8
Tipo de producción: Capítulo de libro **Tipo de soporte:** Libro

3 Lucía Prieto Torres; Rebeca Manso; Deysy Elisabeth Cieza Díaz; Margarita Jo; Linah Kilany Pérez; Társila Montenegro Damaso; Itziar Eraña; Marta Lorda; Dolores Suarez Massa; Salma Machan; Raúl Córdoba; Mariano Ara; Luis Requena; Socorro M Rodriguez Pinilla; Miguel A Piris. Large Cells With CD30 Expression and Hodgkin-like Features in Primary Cutaneous Marginal Zone B-Cell Lymphoma: A Study of 13 Cases.The American journal of surgical pathology. 27/05/2019. ISSN 1532-0979

4 Arantza Onaindia; Sonia González de Villambrosía; Lucía Prieto Torres; Socorro M Rodríguez Pinilla; Santiago Montes Moreno; Carmen González Vela; Miguel A Piris. [DUSP22]-rearranged anaplastic lymphomas are characterized by specific morphological features and a lack of cytotoxic and JAK/STAT surrogate markers.Haematologica. 104 - 4, pp. e158. (Italia): 04/2019. ISSN 1592-8721

5 Deysy E Cieza Díaz; Lucía Prieto Torres; Socorro M Rodríguez Pinilla; Raúl Córdoba Mascuñano; Rebeca Manso Alonso; Salma Machan; Miguel Ángel Piris Pinilla; Luis Requena Caballero. Mycosis Fungoides Associated With Lesions in the Spectrum of Primary Cutaneous CD30+ Lymphoproliferative Disorders: The Same Process or 3 Coexisting Lymphomas?. The American Journal of dermatopathology. 29/03/2019. ISSN 1533-0311

6 Lucia Prieto Torres; Socorro M Rodriguez Pinilla; Arantza Onaindia; Mariano Ara; Luis Requena; Miguel Á Piris. CD30-positive primary cutaneous lymphoproliferative disorders: molecular alterations and targeted therapies.Haematologica. 104 - 2, pp. 226 - 235. (Italia): 02/2019. ISSN 1592-8721

7 Lucía Prieto Torres; Itziar Eraña; Rocio Gil Redondo; Inés Gómez de la Riva; Rebeca Manso; Raquel Pajares; Raúl Córdoba; Salma Machan; Mariano Ara; Luís Requena; Miguel Á Piris; Socorro M Rodríguez Pinilla. The Spectrum of EBV-Positive Mucocutaneous Ulcer: A Study of 9 Cases.The American journal of surgical pathology. 43 - 2, pp. 201 - 210. 02/2019. ISSN 1532-0979

8 Isabel Martínez Pallás; Claudia Conejero Del Mazo; Lucía Prieto Torres. Allergic contact stomatitis caused by propolis candies.Contact dermatitis. 78 - 6, pp. 418 - 419. 06/2018. ISSN 1600-0536

9 Amanda Pereira; Gerardo Ferrara; Paola Calamaro; Carlo Cota; Cesare Massone; Francesca Boggio; Lucia Prieto Torres; Lorenzo Cerroni. The Histopathological Spectrum of Pseudolymphomatous Infiltrates in Cutaneous Lupus Erythematosus.The American Journal of dermatopathology. 40 - 4, pp. 247 - 253. 04/2018. ISSN 1533-0311

10 Francesca Boggio; Viviana Lora; Carlo Cota; Amanda Pereira; Robert Müllegger; Lucia Prieto Torres; Lorenzo Cerroni. Cutaneous hemophagocytosis: Clinicopathologic features of 21 cases.Journal of the American Academy of Dermatology. 78 - 2, pp. 377 - 382. 02/2018. ISSN 1097-6787

11 A Rivera Rodríguez; L Prieto Torres; F Felipo Berlanga; M Ara Martín. Acquired reactive perforating collagenosis associated with Hodgkin disease.Clinical and experimental dermatology. 42 - 8, pp. 934 - 936. 12/2017. ISSN 1365-2230

12 Lucía Prieto Torres; Victoria Alegría Landa; Concepción Llanos; Alicia Córdoba; Heinz Kutzner; Luis Requena. Cutaneous Malignant Melanoma With Rhabdoid Morphology and Smooth Muscle Differentiation: A Challenging Histopathologic Diagnosis.The American Journal of dermatopathology. 39 - 5, pp. 397 - 403. 05/2017. ISSN 1533-0311
Posición de firma: 1
Nº total de autores: 5

13 Victoria Alegría Landa; Margarita Jo Velasco; Lucía Prieto Torres; Luis Requena. Genital folliculo-sebaceous cystic hamartoma: A claim of the stroma as a clue in the diagnosis of proliferations with follicular differentiation.Journal of cutaneous pathology. 44 - 5, pp. 504 - 508. 05/2017. ISSN 1600-0560
Posición de firma: 3
Nº total de autores: 4

14 Lucía Prieto Torres; Francesca Boggio; Alexandra Gruber Wackernagel; Lorenzo Cerroni. Nodular Sclerodermatous Chronic Cutaneous Graft-Versus-Host Disease (GvHD): A New Clinicopathological Variant of Cutaneous Sclerodermatous GvHD Resembling Nodular/Keloidal Scleroderma.The American Journal of dermatopathology. 25/04/2017. ISSN 1533-0311
Posición de firma: 1
Nº total de autores: 4

15 S Hernández Ostiz; L Prieto Torres; G Xirotagaros; L Noguera Morel; Á Hernández Martín; A Torrelo. Autoinflammatory Diseases in Pediatric Dermatology-Part 1: Urticaria-like Syndromes, Pustular Syndromes, and Mucocutaneous Ulceration Syndromes.Actas dermo-sifiliograficas. (España): 23/04/2017. ISSN 1578-2190
Posición de firma: 2
Nº total de autores: 6

16 S Hernández Ostiz; G Xirotagaros; L Prieto Torres; L Noguera Morel; A Torrelo. Autoinflammatory Diseases in Pediatric Dermatology-Part 2: Histiocytic, Macrophage Activation, and Vasculitis Syndromes.Actas dermo-sifiliograficas. (España): 21/04/2017. ISSN 1578-2190

17 Álvaro Rivera Rodríguez; Sergio Hernández Ostiz; Ana L Morales Moya; Lucía Prieto Torres; Marcial Álvarez Salafranca; Mariano Ara Martín. [Zosteriform lichen aureus. Pediatric clinical case].Archivos argentinos de pediatria. 115 - 2, pp. e82. (Argentina): 01/04/2017. ISSN 1668-3501
Posición de firma: 4
Nº total de autores: 6

18 Lucia Prieto Torres; Tamara Gracia Cazaña; María Pilar Sánchez Salas; Gorka Muñiz; Esteban Padgett; Yolanda Gilaberte. Unilateral progressive telangiectatic eruption with a metameral distribution during pregnancy.Journal of obstetrics and gynaecology : the journal of the Institute of Obstetrics and Gynaecology. 37 - 3, pp. 377 - 378. 04/2017. ISSN 1364-6893
Posición de firma: 1
Nº total de autores: 6 **Autor de correspondencia:** Si

19 L Prieto Torres; J Sánchez Bernal; T Gracia Cazaña; S Hernández Ostiz; M Ara Martín. [Targetoid bullous lesion in a farmer's hand: A relevant entity to a country doctor].Semergen. 43 - 3, pp. 256 - 257. (España): 04/2017. ISSN 1578-8865
Posición de firma: 1
Nº total de autores: 5

20 Victoria Alegría; Lucía Prieto Torres; Carlos Santonja; Raul Córdoba; Rebeca Manso; Luis Requena; Socorro María Rodríguez Pinilla. MYD88 L265P mutation in cutaneous involvement by Waldenström macroglobulinemia.Journal of cutaneous pathology. 30/03/2017. ISSN 1600-0560
Posición de firma: 2
Nº total de autores: 7 **Autor de correspondencia:** No

21 Lucía Prieto Torres; Patricia Iranzo; Javier Sanchez Bernal; Laura Manuela Murillo Jaso. [Breast carcinoma en cuirasse simulating dermatitis in an octogenarian patient].Revista espanola de geriatria y gerontologia. (España): 15/03/2017. ISSN 1578-1747
Posición de firma: 1
Nº total de autores: 4 **Autor de correspondencia:** Si

22 Lucía Prieto Torres; Javier Sanchez Bernal; Francesc Felipo; Mariano Ara Martín. Verrucous plaque-like lesion with progressive growth in the scalp of a 3-month-old infant.Enfermedades infecciosas y microbiologia clinica. (España): 08/03/2017. ISSN 1578-1852
Posición de firma: 1
Nº total de autores: 4

23 L Prieto Torres; T Gracia Cazaña; I Pastushenko; A L Morales Moya; J Soria; M Ara Martín. [Bullous haemorrhagic dermatosis at distant sites due to enoxaparin: An uncommon secondary effect in an anticoagulated oncology patient].Semergen. 42 - 7, pp. 504 - 506. (España): 10/2016. ISSN 1578-8865
Posición de firma: 1
Nº total de autores: 6

24 Ievgenia Pastushenko; Gert G Van den Eynden; Sandra Vicente Arregui; Lucia Prieto Torres; Ramiro Alvarez Alegret; Ignacio Querol; Luc Y Dirix; Francisco J Carapeto; Peter B Vermeulen; Steven J Van Laere. Increased Angiogenesis and Lymphangiogenesis in Metastatic Sentinel Lymph Nodes Is Associated With Nonsentinel Lymph Node Involvement and Distant Metastasis in Patients With Melanoma.The American Journal of dermatopathology. 38 - 5, pp. 338 - 346. 05/2016. ISSN 1533-0311
Posición de firma: 4
Nº total de autores: 10

25 L Prieto Torres; S Hernández Ostiz; E Pelegrina Fernández; C Conejero Del Mazo. The Role of Epidermal Stem Cells in the Origin of Basal Cell Carcinoma.Actas dermo-sifiliograficas. 107 - 4, pp. 341 - 342. (España): 05/2016. ISSN 1578-2190
Posición de firma: 1
Nº total de autores: 4 **Autor de correspondencia:** Si

26 Hernández-Ostiz S, Prieto-Torres L, Morales-Moya AL, Ara-Martín M. Escabiosis costrosa, reto diagnóstico y terapéutico.Piel. Formación continuada en dermatología. 31, pp. 328 - 332. 26/04/2016. ISSN 0213-9251
Tipo de soporte: Revista
Posición de firma: 2
Nº total de autores: 4

27 L Prieto Torres; M Llamas Velasco; S Machan; R Haro; S de Asis; M Carmo; A Loredo; C del Puerto; I Fried; W Kempf; L Cerroni; L Requena. Taxanes-induced cutaneous eruption: another histopathologic mimicker of malignancy.Journal of the European Academy of Dermatology and Venereology : JEADV. 30 - 4, pp. 638 - 644. 04/2016. ISSN 1468-3083
Posición de firma: 1
Nº total de autores: 12

28 C Bernárdez; L Prieto Torres; E Macías; J L Ramírez Bellver; R Haro Ramos; J L Diaz Recuero; L Requena. Concurrent presentation of cutaneous lesions of deep linear morphoea and discoid lupus erythematosus.Lupus. 25 - 2, pp. 204 - 208. 02/2016. ISSN 1477-0962
Posición de firma: 2
Nº total de autores: 7

29 Prieto-Torres L, Torrelo A.Dermatitis atópica y otras erupciones eccematosas. Pediatr Integral. XX - 4, pp. 216 - 226. 2016. ISSN 1135-4542
Tipo de soporte: Revista
Posición de firma: 1
Nº total de autores: 2 **Autor de correspondencia:** Si

30 I Pastushenko; L Prieto Torres; Y Gilaberte; C Blanpain. Skin Stem Cells: At the Frontier Between the Laboratory and Clinical Practice. Part 1: Epidermal Stem Cells.Actas dermo-sifiliograficas. 106 - 9, pp. 725 - 732. (España): 11/2015. ISSN 1578-2190
Posición de firma: 2
Nº total de autores: 4

31 Prieto-Torres L; Alegría-Landa V; Morales-Moya AL; E-E; Ara-Martín M; Requena L. Lupus panniculitis refractory to multiple therapies treated successfully with Rituximab: A case report and literature review. Australasian Journal of Dermatology. ISSN 1440-0960
Tipo de soporte: Revista
Posición de firma: 1
Nº total de autores: 6 **Autor de correspondencia:** Si
Resultados relevantes: Aceptado pendiente de publicación.Article DOI: 10.1111/ajd.12685

32 Lucla Prieto Torres; Claudia Bernárdez; Sergio Hernández Ostiz; Ievgenia Pastushenko; Mariano Ara Martin; Luis Requena. Necrobiosis lipoidica developing within a surgical scar in a non-diabetic patient: Type III Koebner phenomenon (isomorphic response), Wolf's isotopic response or Ruocco's immunocompromised cutaneous district?. Indian journal of dermatology, venereology and leprology. 83 - 2, pp. 233 - 236. (India): ISSN 0973-3922
Posición de firma: 1
Nº total de autores: 6

33 Lucía Prieto Torres; David Lapeña Gil; Francesc Felipo; Mariano Ara Martín. Papulosis linfomatoide angioinvasiva (tipo E) con diseminación intralinfática: Un simulador histológico de linfomas agresivos. Piel. Formación continuada en dermatología. ISSN 0213-9251
Tipo de soporte: Revista
Posición de firma: 1
Nº total de autores: 4 **Autor de correspondencia:** Si
Resultados relevantes: Aceptado pendiente de publicación


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